PROGRESS REPORT -- 1998

CENTER FOR MEDICAL, AGRICULTURAL AND VETERINARY ENTOMOLOGY

AGRICULTURAL RESEARCH SERVICE

U.S. DEPARTMENT OF AGRICULTURE

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The **Center for Medical, Agricultural and Veterinary Entomology (CMAVE)** is the only ARS-USDA research center devoted exclusively to entomology. This annual report provides abstracts of research in progress and is not intended for citation in any publication. Reprints of published articles may be obtained by writing the individual authors.

MISSION STATEMENT

The Center will conduct research on insects of agricultural, medical and veterinary importance with the goal of achieving control of pest species through the development of environmentally acceptable approaches. Emphasis is placed on developing components and systems for integrated pest management, based upon an understanding of the behavior, physiology and ecology of pest species. Sensitive detection devices that employ semio-chemicals and electronic technology will provide the means for early intervention. Investigations will lead to biological control based on parasites, predators and microbes, and thus provide alternative biorational tools for managing populations of pest species. Special attention is focused on insect pests of field and horticultural crops, stored products and on arthropod pests of medical and veterinary importance. Protection of humans from arthropods of medical importance is a continuing priority. The scope of the Center's research is national and international and impacts agricultural production, postharvest storage and transport of agricultural commodities, and protection from household and disease carrying arthropods. Research is conducted to meet the needs of state and federal regulatory agencies, the Department of Defense, industry, universities, growers, commodity groups and the public at large.

STAFF AND ORGANIZATIONAL CHANGES

We are pleased to announce the addition of a new entomologist to our permanent scientific staff. Dr. David Oi is conducting research in the Imported Fire Ants and Household Insects Unit. We also are pleased to announce the participation of CMAVE in the new Center for Biological Control Research and Education at Florida A&M University in Tallahassee, Florida. In addition, Ms. Carroll Godfrey retired as Administrative Officer, and has been replaced by Ms. Jacquelyn Sullivan.

HONORS

A number of individuals at CMAVE received significant awards during 1998, as follows: Ms. Bonnie Bayer was selected as Secretary of the Year for the South Atlantic Area; Mr. Robert Heath received a Technology Transfer award for developing sensitive detection systems for the Mediterranean and Mexican fruit flies; Dr. Herbert Oberlander was selected for a U. S. Government Presidential Rank Award for his research in insect tissue culture and leadership of the ARS laboratories in Gainesville; Dr. John Sivinski was honored as part of the ARS-APHIS Medfly Group that received a USDA Honor Award for Excellence; and Dr. James Tumlinson was inducted into the Agricultural Research Service Hall of Fame for his many achievements in the area of chemical communications in insects.

Herbert Oberlander
Center Director

TABLE OF CONTENTS

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H. Oberlander, Center Director

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This Research Unit describes, analyzes and manipulates insect behaviors that are responsible for visual and chemical stimuli that regulate reproduction, feeding, foraging and migration. Principles of behavior are emphasized, especially reproductive behavior of pest and beneficial insects. Results of this research are applied directly to control programs and technology, such as genetic eradication programs against Mediterranean fruit flies in Central America and Caribbean fruit flies in Florida, and integrated pest management of lepidopterous pests of field and vegetable crops.

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This Research Unit investigates the chemical, biochemical and physiological factors that regulate insect behavior and the interaction of insects with plants and other organisms in the environment. The research program focuses on the following major areas: identification, synthesis, and behavioral evaluation of pheromones that regulate mating and other behaviors of important insect pests; identification, synthesis and behavioral evaluation of kairomones and other semiochemicals that influence the foraging behavior of beneficial entomophagous insects; identification, synthesis and behavioral evaluation of plant-produced chemicals that influence the behavior of insects; and elucidation of the biochemical mechanisms that regulate insect pheromone production, release and perception.

Imported Fire Ant and Household Insects

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This Research Unit develops reduced-risk integrated management strategies for cockroaches and their attendant allergens, pest ants, fire ants and, through cooperative agreements, termites. Areas of research include insecticide detoxification mechanisms; spatially-based risk assessment and insect behavioral ecology pertaining to the development of baits, repellents, and biological control agents; population dynamics; sociobiology of insects; bioecology and biodiversity; and pheromone chemistry and chemical ecology.

Mosquito and Fly

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Research in this Unit results in new technology that provides the basis for integrated management of mosquitoes and filth flies. Recipients and end-users of Unit research include livestock producers; animal, public, and vector abatement organizations; military personnel; and the public. Specific areas of research include: the biological control of mosquitoes and flies (microbial pathogens, parasites, parasitoids); the regulation of fly populations via manipulation of host attraction, host selection, and blood feeding factors; the development of personal protection technology, including the discovery of new mosquito attractants and repellents; and the discovery and use of genetic, biochemical, and physiological factors as regulating mechanisms for populations of mosquitoes and flies.

Postharvest and Bioregulation

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This Research Unit conducts research on the detection, population estimation and control of stored product insects. New detection tools are developed based on acoustical and electronic methods, as well as chemical ecology. Research approaches to population management include the application of insect behavior, molecular biology, biochemistry and tissue culture to the control of growth and development of these insects.

EXAMPLES OF RECENT RESEARCH

Parasites Smell Success

Parasites and predatory arthropods often prevent plants from being severely damaged by killing herbivores as they feed on the plants. A breakthrough in understanding how these biological control agents locate their insect hosts was achieved with the isolation and identification of a volatile chemical, "volicitin", obtained from the oral secretions of beet armyworm caterpillars. When applied to damaged leaves of corn seedlings, volicitin induced the seedlings to emit volatile compounds that attract parasitic wasps which are natural enemies of the caterpillars.

Fruit Flies Find New Trap Alluring

A trapping system based on a new 3-component lure, has been developed for the Mediterranean fruit fly. It has been tested successfully in seven foreign countries. It was also highly effective during a recent Medfly eradication program in Tampa, Florida. An improved lure and trapping system has also been developed for the Caribbean fruit fly.

Genes on the Move

The ability to insert genes into *Drosophila* suggested opportunities for a new approach to insect control. Progress in this area, however, has been held up by a lack of genetic transposons that would allow scientists to insert genes of choice in other insect species of economic importance. Recent experiments with both fruit flies and moths are showing promise. There is new evidence that a new transposon, *piggybac*, will function in both the Indianmeal Moth and the Mediterranean fruit fly, while another transposable element, *hopper,* was isolated from the Oriental fruit fly.

Taking a Bite out of Fire Ants

When Imported fire ants were introduced into the United States, almost all of their natural enemies remained behind in South America. Efforts to introduce biological control of fire ants have led to the first release in the United States of a South American phorid fly. Fly eggs hatch into larvae in the fire ants, and have the peculiar effect of decapitating the host. Then, the flies complete their development in the severed head capsule until they emerge as adult flies.

Houseflies Succumb to Worms

Adult houseflies that develop from larvae infected with parasitic nematodes lived only half as long as uninfected flies. This nematode species, originally collected from Brazil, has potential as a biological control agent for houseflies because it appears to be host specific and can be raised easily in large quantities. Moreover, because there are few natural enemies that attack flies in the larval stage this nematode may be compatible with other biological control agents.

Eavesdropping on Insects

Highly sensitive methods have been developed for detection of hidden infestations of insects in stored grain based on the sounds that are made as the larvae feed. Field trials indicate practical potential for using a sampling system with sound detectors for quantitiative sampling of hidden infestations. In addition, a commerical grain probe trap was modified by incorporating a sensor head with infrared electronics so that insects that enter the trap can be electronically sensed and counted. These detection methods may be applicable to a wide variety of insects.

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BEHAVIOR

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CRIS - 6615-22000-011-00D--Behavioral Ecology and Management of Crop Insect Pests with Semiochemicals

CRIS - 6615-22000-013-00D--Insect Biological Control Through Behavioral and Genetic Manipulation

CRIS - 6615-22000-014-00D--Biological Control Through Artificial Rearing of Natural Enemies and Manipulation of Host Plant Resistance

EVALUATION OF OVIPOSITION STIMULANTS AS ARTIFICIAL DIET AMENDMENTS FOR THE PEPPER WEEVIL

D.L. Johanowicz[1] and E.R. Mitchell

Objective: Feeding damage by the pepper weevil, *Anthonomus eugenii* Cano, can limit the production of peppers in the southern United States. Infested blossom buds and immature peppers may prematurely fall off the plant, or mature peppers may contain frass or decayed plant tissue. To alleviate the costs and other impacts due to chemical control of the weevil, augmentative biological control with a *Catolaccus* sp. is proposed. In order to rear large numbers of these parasitoids, large numbers of pepper weevils are necessary. Rearing the pepper weevil on artificial diet rather than host plant material would be more efficient, but the females will not oviposit into the diet. Therefore, our goal is to determine whether we can stimulate the pepper weevil to oviposit into something other than their natural host plant in efforts to eventually rear them entirely on artificial diet. Adding potential stimulants to an inert medium is the first step in determining the modifications necessary to make artificial diet an acceptable food and oviposition substrate.

Methods: Pepper plant tissues (flowers, pericarp, leaves, sprouts) from different pepper cultivars were macerated in 1 cc of water, filtered, and applied to filter paper (Whatman No.1). The filter paper then was folded to form a sphere, and wrapped in stretched Parafilm. Pepper leaves of a similar size to those which were macerated were also formed into a sphere and wrapped in Parafilm as a positive control, and filter paper treated with water served as a negative control. These oviposition devices were presented to a known number of 10-20 day old females in a no-choice design. The females were sexed according to metatibial mucrone and genital characters. The females were supplied with a honey-sucrose-water solution throughout the experiments. Two days after presentation, the oviposition devices were dissected under a compound microscope, and the number of eggs counted.

Results: Preliminary results indicate that there are extractable components in pepper plants which stimulate oviposition into an inert substrate, and that plant age may alter the nature of the stimulating components.

[1] University of Florida

FACTORS WHICH INFLUENCE THE PROGENY SEX RATIO OF *Diadegma insulare,* A PARASITOID OF THE DIAMONDBACK MOTH

D.L. Johanowicz[1] and E.R. Mitchell

Objective: One important aspect of an augmentative biological control program is to rear ample numbers of female parasitoids for field releases. The parasitoid *Diadegma insulare* currently is being reared for releases to help manage diamondback moth populations. The current rearing system yields a male-biased sex ratio, which can be problematic when large numbers of females are necessary for successful field releases. The objective of the present study is to evaluate factors which influence the progeny sex ratio of *D. insulare*. One factor under investigation is the age at which the parasitoids are exposed to their hosts.

Methods: Upon emergence, adult *D. insulare* were removed from emergence cages and placed into new cages without hosts, five pairs per cage, for one, three, and five days. The one-, three- and five-day old wasps were provisioned with honey and water prior to and during exposure to hosts. All wasps then were exposed to 250 late second to late third instar diamondback moth larvae. Fresh collards were provided for the hosts throughout the study. After 48 hours of exposure to hosts, the wasps were removed, and the hosts were allowed to develop to adults. The resulting numbers of adult diamondback moths, *D. insulare* males, and *D. insulare* females per treatment were recorded and compared.

Results: Preliminary results indicate that the females withheld from hosts for 5 days produce more female progeny than the females withheld from hosts for one and three days, although all conditions still produce a male-biased sex ratio.

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MORPHOLOGICAL COMPARISONS OF IMMATURE STAGES OF TWO PARASITOIDS OF DIAMONDBACK MOTH

G.Y. Hu[1], E.R. Mitchell, and D. Johanowicz[1]

Objective: *Diadegma insulare* is a parasitoid of the diamondback moth in cabbage, collard, and other cole crops. This biological control agent is native to the U.S. and South American Countries. Recently, an exotic parasitoid (originated from Asia), *Cotesia plutellae*, has been released into cabbage fields in these areas for control of diamondback moth. No data was available on how to separate the two parasitoids in their immature stages. The objective of this study was to determine morphological differences of immature stages between the two parasitoids.

Methods: The native parasitoid, *D. insulare,* was collected from cabbage in Bunnell, FL, in May 1996 and colonized in our laboratory. The exotic parasitoid, *C. plutellae,* originally from Southeastern Asia, was purchased from BioFac, Mathis, TX, and was colonized similarly in our laboratory. The two parasitoids were reared on diamondback moth larvae fed on collard leaves. The parasitoids and diamondback moth larvae were maintained under a 12:12 h L:D cycle at 25 °C and 50% RH. Eggs and larvae of both parasitoids were dissected from diamondback moth larvae and were compared morphologically under a microscope. Cocoons were collected and compared by observation.

Results: *Diadegma insulare* attacked all instars of diamondback moth larvae, but the parasitoid larvae did not complete development if the adult wasps attacked early instars of the host. On the contrary, *C. plutellae* preferred early instars of the host, and the larva finished its growth before diamondback moth larvae completed development. Eggs of both parasitoids require 2-3 days to hatch. They are transparent in color and slightly crescent-shaped. *Cotesia* eggs have one end (anterior) blunt, and the other pointed with a spiny tail (Fig. 1, left), but both ends of *Diadegma* eggs are blunt (Fig. 1, right). Larvae of both parasitoids go through four instars, and need 8-12 days to complete development. They are cream-colored, and worm-like, with the anterior end wider than the posterior end. *Cotesia* larvae have a bulb-like first segment (head) (Fig. 2). *Diadegma* larvae, however, are tapered toward the posterior end. The last segment extends to a pointed tail that occupies 1/6 of the body length in the 1st and 2nd instars (Fig. 3), but it becomes very short in the 3rd and 4th instars. *Cotesia* cocoons are white in color, and are separated from its host (Fig. 4). Cocoons of *Diadegma,* on the other hand, are light-yellow in color, and are formed within the host cocoon (Fig. 5).

Our results show that eggs, larvae, and cocoons of the two parasitoids attacking diamondback moth larvae are distinguishable based on morphological features. These data can be useful in evaluating parasitism contributed by these parasitoids in biological control programs.

[1] University of Florida.

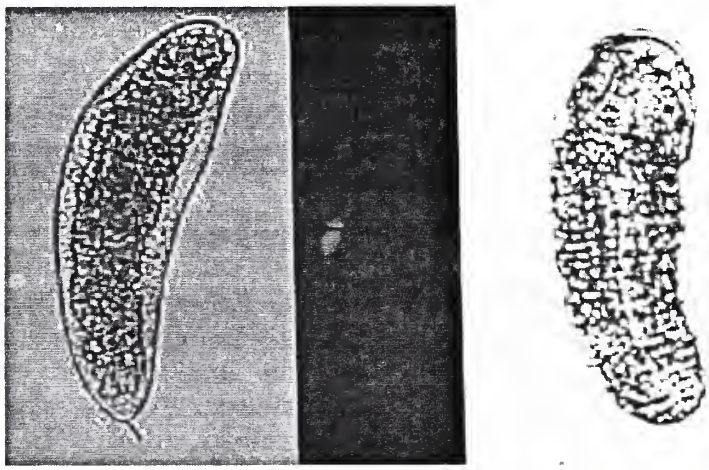

Fig.1. left: *Cotesia* egg, right: *Diadegma* egg

Fig. 2. *Cotesia* 2nd instar larva

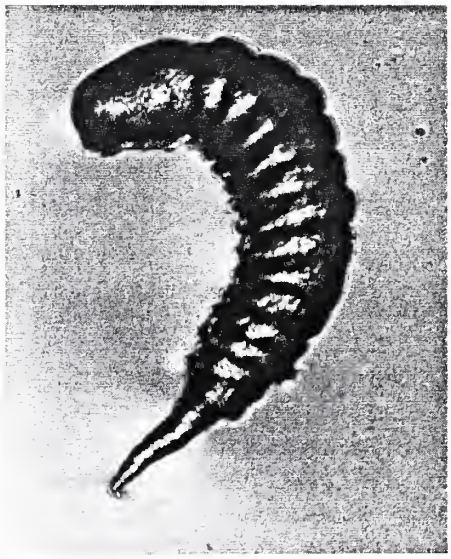

Fig 3. *Diadegma* 2nd instar larva

Fig.4. *Cotesia* cocoon

Fig. 5. *Diadegma* cocoon

CRIS 6615-22000-011-00D

COLLECTION OF FALL ARMYWORM (LEPIDOPTERA: NOCTUIDAE) USING DIFFERENT PHEROMONE SOURCES AND TRAP TYPES

R.L. Meagher, Jr.

Objective. The objective these studies was to compare pheromone lures and trap designs in the collection of fall armyworm moths under field conditions. Comparisons of moth capture were made among Unitraps (bucket traps) and fabric mesh cone traps (*Heliothis* traps) in one experiment, whereas comparisons of moth capture were made among three Unitrap colors in another experiment. Additionally, observations of moth behavior were conducted under field conditions to compare trap efficiency (number of moths collected vs. number of moths attracted).

Methods. All white Unitraps (white canopy, funnel, and bucket) were set out in early April, 1998, in northwestern Alachua County, Florida to capture male fall armyworm. This part of the county was planted to over 470 ha of silage corn and offered fields separated by paved and unpaved roads and forested strips. The experiment contained four treatments: Unitraps with either Trécé® red septa lures or Scentry® gray septa *S. frugiperda* sex pheromone lures, and *Heliothis* double-cone traps with either lure. Traps were placed on 1.5-m metal poles along pivot roads and edges in one 80 ha field. Trapping began 8 April and ended 12 June. Moth numbers per night were compared across treatments using analysis of variance. Peanuts ('Georgia Green') were planted in the same areas during summer and fall 1998. This experiment was designed to compare moth capture using three Unitrap colors (all white, all green, or standard = green canopy, yellow funnel, white bucket). Trapping began 21 July and ended 30 September.

Moth behavior was observed on six separate nights between 2030 and 2330 in both corn and peanut fields. Unitraps and *Heliothis* traps, baited with pheromone lures, were placed on 2.1-m metal poles. Observers were positioned so that they could see moths attracted to the traps. The number of moths observed within ≈ 0.3 m of the traps were counted. At evening's end, all captured moths were noted and trap efficiency was recorded as number of moths captured divided by number of moths attracted to traps.

Results. In the experiment conducted in corn, Unitraps baited with Trécé lures collected the highest number of moths (Fig. 1). When averaged across the season, both pheromone lures collected similar numbers of moths. However, Unitraps collected more moths than *Heliothis* cone traps (7.7 per night compared to 2.4, respectively). Observations of fall armyworm behavior at night showed that the *Heliothis* cone traps caught only 19.3% of moths attracted to the trap, whereas Unitraps collected only 16.0%. There was a large variation in trap efficiency among nights, and it appears that the cone traps can more effectively collect moths when there are large populations. However, moths in the cone traps were better able to escape during the night so that observations made in the morning showed low numbers of moths collected.

In the experiment conducted in peanuts, yellow Unitraps (54.0 moths per night) collected more moths than white traps (24.0), which collected more moths than green traps (11.9) (Fig. 2).

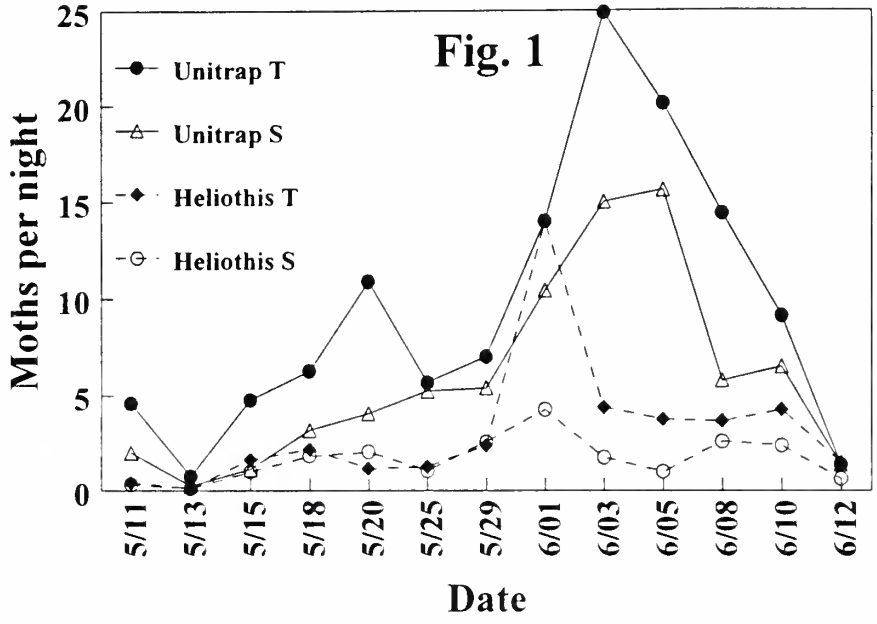
Fig. 1
Unitrap T
Unitrap S
Heliothis T
Heliothis S
Moths per night
25
20
15
10
5
0
5/11
5/13
5/15
5/18
5/20
5/25
5/29
6/01
6/03
6/05
6/08
6/10
6/12
Date

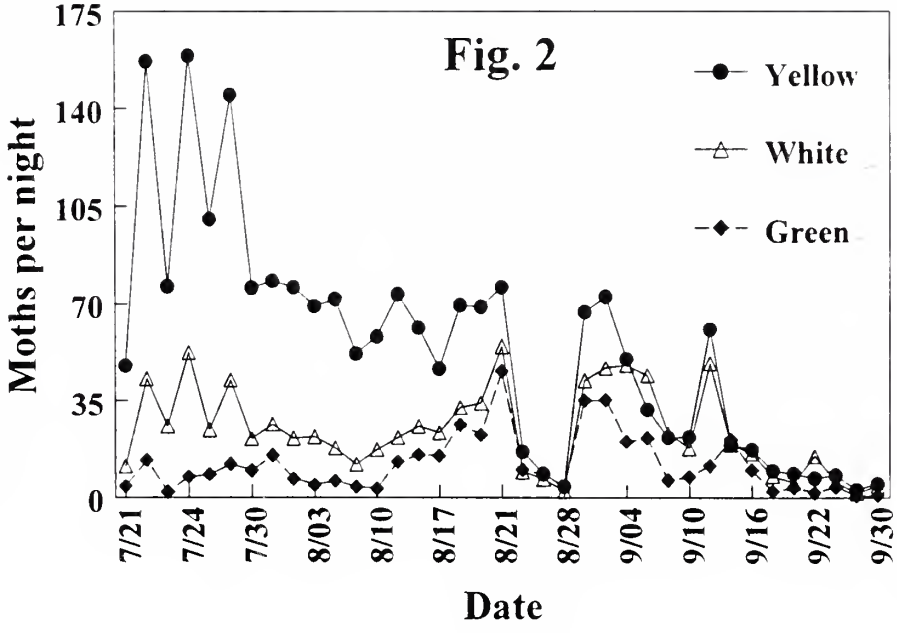
Fig. 2
Yellow
White
Green
Moths per night
175
140
105
70
35
0
7/21
7/24
7/30
8/03
8/10
8/17
8/21
8/28
9/04
9/10
9/16
9/22
9/30
Date

CRIS 6615-22000-011-00D

EARLY-SEASON AUGMENTATIVE RELEASES OF A NATIVE PARASITOID FOR CONTROL OF DIAMONDBACK MOTH IN CABBAGE

E.R. Mitchell, G.Y. Hu[1] , and D. Johanowicz[1]

Objective: *Diadegma insulare* is an important parasitoid that suppresses diamondback moth (DBM) populations in North America. However, our previous studies showed that the field populations of this parasitoid did not increase until March of the spring season in northeast Florida. The objective of this study was to evaluate parasitism of the DBMafter releasing *D. insulare* in cabbage early in the spring growing season.

Methods: *Diadegma insulare* were collected from cabbage in Bunnell, Florida, in May of 1996 and were colonized in our laboratory since then. The parasitoids were reared on DBM larvae that fed on artificial diet. Parasitoid and DBM colonies were maintained under a 12:12 h L: D cycle at 25 °C. and 50% RH. Adult wasps less than 4-d old were collected from the colony for releases. Our previous study showed that when female *D. insulare* were preconditioned by exposing them to plant material with DBM larval feeding damage, more responded to the plant-larval complex than naive wasps. Therefore, we used this information in our field release program and preconditioned *Diadegma* in screened cages with the plant-larval complex prior to releases. A total of three fields totaling 59 acres (7, 22, and 30 acres for fields A, B, and C, respectively) was used for releases. The release rate was 100 pairs per acre, and three to four releases were made in each field in February and early March 1998. Diamondback moth larvae were collected from cabbage plants in release and control fields (no releases made) and dissected under a microscope for parasitism.

Results: Field A showed no parasitism of DBM larvae by *Diadegma insulare* before releasing the parasitoid. The larval parasitism increased to 52% one week after the first release (February 25), fluctuated from 30% to 50% for four weeks, and then declined quickly. Field B had 33% larval parasitism of DBM by *D. insulare* before parasitoid releases. Parasitism in field B increased to 67% one week after the first release (March 3), remained between 33% and 53% for five weeks, and then declined quickly. Data on parasitism of DBM larvae before releasing *Diadegma* was not available for field C. Parasitism by *Diadegma* in field C ranged from 35% to 45% for five weeks after the first release (February 12), and then declined quickly. Data from control fields (no parasitoid releases) were similar to that from the release fields. This may be because the control fields were not far enough away from the release fields, and *Diadegma* possibly spread into the control fields soon after they were released. Rapid declines of parasitism in late March and early April were due to pesticide applications by the growers. Lannate and Spintor were applied at the time of declining populations.

Studies conducted during the past five years in the same commercial cabbage production area showed that parasitism of DBM larvae increased to a high of only 45% in late March and early April, but results of this study showed that releasing *Diadegma* in early spring helped to build up and maintain populations of the parasitoids earlier than normal in the season. The level of parasitism possibly would have remained high during the entire season if no pesticides had been applied. Early-season augmentation of this native parasitoid may enhance biological control of DBM in northeast Florida.

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MANAGEMENT OF DIAMONDBACK MOTH IN CABBAGE USING COLLARDS AS A TRAP CROP

E.R. Mitchell, G.Y. Hu[1], and D.L. Johanowicz[1]

Objective: The diamondback moth (*Plutella xylostella* L.) is a worldwide pest of cruciferous vegetables, including cabbage, greens, broccoli, and cauliflower. Because of concerns with pesticide safety, as well as the high degree of pesticide resistance found in the diamondback moth, an integrated pest management (IPM) approach is under investigation. Previous studies indicate that the diamondback moth invades fields from the margins, so a trap crop system can be designed by planting collard greens, a favored host plant, in the margins of the cabbage fields. The objective of this study was to investigate the effectiveness of collard trap crops by assessing larval counts, the damage to, and the marketability of the cabbage grown under the different conditions.

Methods: A total of twelve commercial cabbage fields, six in Hastings, Florida (Col1, Col2, Col3, and Cab1, Cab2, Cab3) and six in Bunnell, Florida (Col4, Col5, Col6, and Cab4, Cab5, Cab6) was studied. In each area, three fields were planted with collard trap crops (Col) and three fields were planted with cabbage alone (Cab). Two rows of collards were planted along the two peripheral rows and seven collard plants were planted on the ends of each row. Diamondback moth larvae were sampled weekly throughout the growing season. The mean numbers of diamondback moth larvae in the cabbage grown in all fields were compared. When possible, cabbage damage ratings were assessed immediately prior to harvest, based on a scale of 1-6; ratings of 1-3 (no damage to head) are generally considered marketable. The mean damage ratings were combined per grower and compared, and the resulting proportions of marketable heads were calculated and compared. After harvest, the growers reported the number of pesticide sprays applied during the course of the study.

Results: Our results showed that collards were an effective trap crop, maintaining larval counts in the surrounded cabbage at or below larval counts in unsurrounded cabbage, even with fewer pesticide treatments. Only in weeks 9-13 were the larval counts between some of the fields different. One unsurrounded field, Cab4 (which we were unable to rate), had higher larval counts than almost all other fields on one sampling date, yet it was sprayed 14 times during the growing season. In only one case did a field with collards have higher larvae counts than the other fields. This field (Col3) was incompletely surrounded by collards. When the cabbage damage ratings and marketability were analyzed, the incompletely surrounded field was the only one with significantly higher damage ratings (Figure 1, Grower 1B) and sustained significantly reduced marketability of cabbage heads (Figure 2) compared to the fields completely surrounded by collards and the conventionally-treated fields. The growers were pleased with the results and will continue using collard trap crops in the future.

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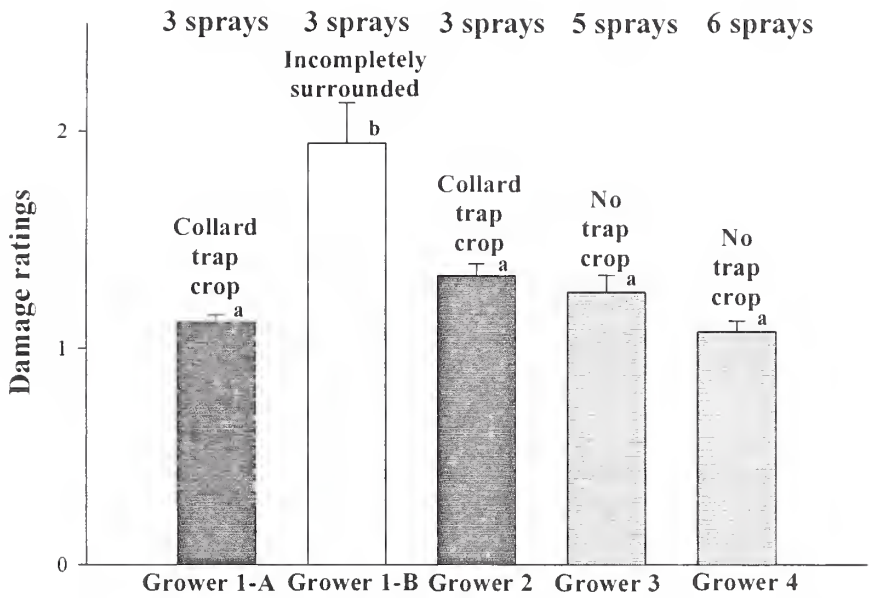

Figure 1. Damage ratings. The bars with same letters indicate no significant differences in damage ratings.

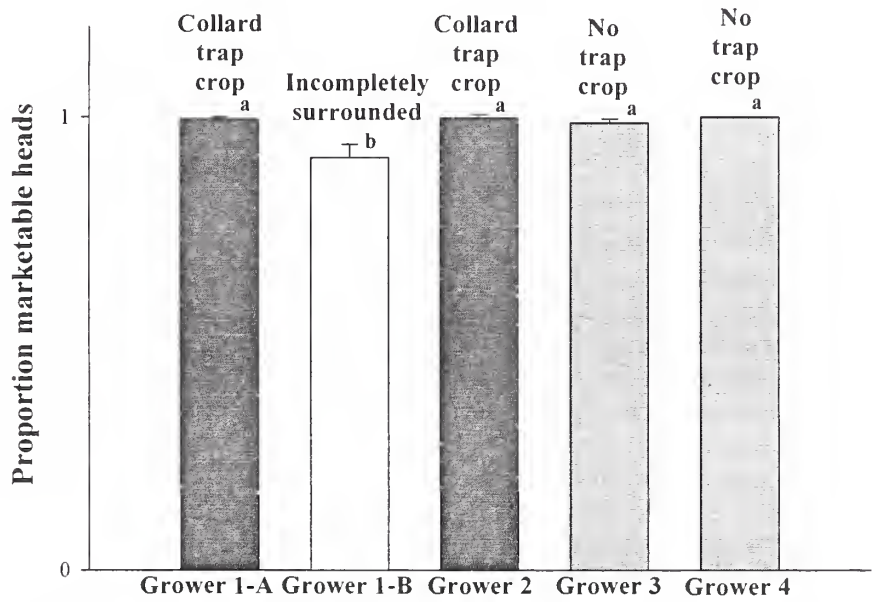

Figure 2. Proportion of marketable heads. The bars with same letters indicate no significant differences in marketable heads.

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ATTRACTION OF COTTON LEAFWORM TO FERMENTATION PRODUCTS FROM BLACKSTRAP MOLASSES PLUS 3-METHYL-1-BUTANOL

E.R. Mitchell, M.S. Mayer, and P.S. Landolt[1]

Objective: To identify non-pheromone chemicals attractive to lepidopterous pests of field and vegetable crops with emphasis on discovering volatile compounds attractive to female moths.

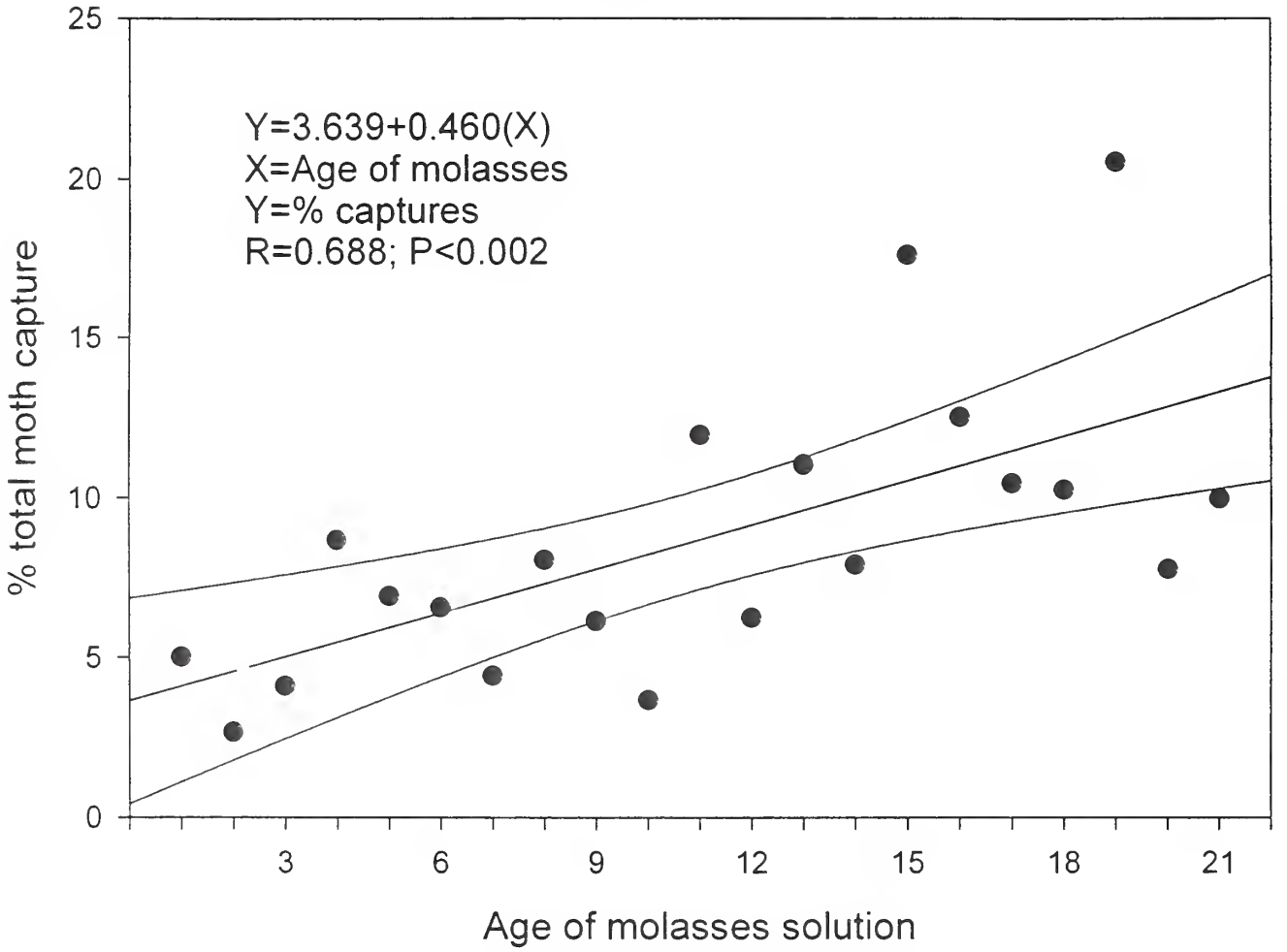
Methods; Unsulphured blackstrap molasses and tap water were mixed (2.5% molasses) and held in open, 3.78 liter plastic containers under a hood in the laboratory (23.3 °C.) for 0, 2, 4, 6, 10, 12, 14, 17, and 19 days. The aged solutions then were set out in the field in Trappit dome traps (Agrisense, Fresno, CA). Each trap well contained 250 ml of molasses solution plus a few drops of non-perfumed detergent. In addition, each trap was baited with a 21 mm polyethylene bottle cap containing 1 ml of 3-methyl-1-butanol (98.5+% pure, HPLC grade). A pin glued to the inside top of the dome was used to secure in place the bottle cap containing 3-methyl-1-butanol.

Treatments were arranged in 3 randomized complete blocks with traps spaced 15 m apart in rows. The traps were positioned on the periphery of separate cotton fields near Trenton, FL. Insects were collected every 1-2 days, after which the traps were re-randomized. The molasses solutions were topped off as needed with originally prepared materials to maintain the prescribed quantity of liquid/trap. In addition, fresh (0-d old solutions) were added to the trial on 15, 16, and 17 September. Bait solutions older than 21 d were routinely discarded due to the limited number of traps available. The insects collected were bagged and returned to the laboratory for identification and determination of mating status of captured females. The test was initiated 14 September and terminated 25 September. Because the molasses solutions continued to age in the field, care was taken to ensure that moth capture data for any particular initial age grouping was adjusted for days post-deployment in the field. For example, the initially deployed 2-d old molasses solution set out 14 September was classed as 13-d old on the final collection date, 25 September. Moth captures for the 3 replicates were totaled for each collection period, and the data were converted to percentage of total moth capture in each age grouping. The data then were subjected to regression analysis. For comparison purposes, 3 bucket traps baited with a commercial lure for cotton leafworm, *Alabama argillacea*, were maintained near the test sites.

Results; The cotton leafworm (CLW), recently has become a recurrent late-season pest on cotton in north-central Florida. The 3 bucket traps baited with CLW lures were operated a total of 247 trap nights from 19 June through 25 September. During this period, not a single male CLW moth was captured in the lure-baited traps. By contrast, the molasses-baited dome traps with 3-methyl-1-butanol captured a total of 782 moths of which 67.9% were females and 32.1% were males. Most (79.7%) females had mated at least one time. There also was a significant linear relationship between age of the molasses solution and moth capture (Fig. 1) with the older solutions capturing a higher percentage of moths than newer, i.e., fresher, solutions.

[1]ARS USDA, Wapato, WA

Fig. 1. Capture of cotton leafworm moths in traps baited with molasses plus 3-methyl-1-butanol.



CRIS: 6615-22000-011-00D

RESPONSE FALL ARMYWORM TO DIFFERENT CONCENTRATIONS OF ACETIC ACID IN WATER PLUS 3-METHYL-1-BUTANOL

E.R. Mitchell, M.S. Mayer, and P.S. Landolt[1]

Objective: To determine the optimum level of glacial acetic acid in water for capture of fall armyworm moths.

Methods: Glacial acetic acid (HPLC grade, 99.7% purity, Fisher Scientific Co.) was admixed with tap water (vol/vol) to yield 5 different concentrations of acetic acid (AA): 0.0625, 0.125, 0.25, 0.5, and 10.0%. The solutions were applied in Trappit dome traps (Agrisense, Fresno, CA) at the rate of 250 ml solution per trap. A few drops of non-perfumed detergent was added to the AA solution to aid capture of moths entering the trap. In addition to the AA solution, each trap was baited with a 21 mm polyethylene bottle cap (P/N 25010008A, Kimble Glass, Inc., Vineland, NJ) containing 1 ml of 3-methyl-1-butanol, 98.5+% pure, HPLC grade (Sigma-Aldrich Chemical Co., Milwaukee, WI). In addition to the AA solutions, the test included two controls: a trap baited with water/detergent solution plus a cap with 3-methyl-1-butanol and also a trap baited with 10% molasses/water solution (vol/vol) plus detergent but no plastic cap with alcohol.

The treatments (5 AA solutions plus the water and molasses controls) were set out in 2 randomized complete blocks adjacent to corn fields near Alachua, FL. The traps were spaced 15 m apart in rows. The test was initiated 7 July and terminated 2 weeks later. Insects were collected every 1-2 days after which the treatments were re-randomized. The trapped insects were bagged and returned to the laboratory for identification and counting.

For statistical analysis, the data were converted to square root X + 0.5 and subjected to ANOVA (SAS, PROC GLM and options) using a split-plot design with blocks and treatments as main plot effects and dates as subplot error. Individual means were separated by Duncan's multiple range test.

Results; A total of 147 fall armyworm moths were captured of which 70.7% were recorded in traps baited with the 10% acetic acid solution + 3-methyl-1-butanol (mean = 6.8 ± 2.7, significantly different from all other treatments, $P<0.0183$, 32 df). There was no significant difference in the number of moths captured among the other 6 treatments. There clearly was no evide .ce of a dose response to AA among the range of concentrations tested.

Of the 104 fall armyworm moths captured in the AA + alcohol treatment, 93.2% were females of which most had mated at least one time (85.5%) as shown by the presence of a spermatophore in the bursa copulatrix. Mating among females caught in the 6 other treatments was 80.6%.

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CRIS: 6615-22000-011-00D

RESPONSE OF FALL ARMYWORM TO DIFFERENT CONCENTRATIONS OF MOLASSES IN WATER PLUS 3-METHYL-1-BUTANOL

E.R. Mitchell, M.S. Mayer, and P.S. Landolt[1]

Objective: To determine the optimum level of molasses in water for capture of fall armyworm moths.

Methods: Unsulphured molasses was mixed with tap water (vol/vol) to yield 5 different concentrations: 0.5, 1.5, 2.5, 5.0, and 10.0% molasses solution, respectively. The solutions were applied in Agrisense Trappit Dome Traps (Great Lakes IPM, Vestaburg, MI) at the rate of 250 ml solution per trap. A small quantity of Triton X-100 spreader-sticker was added to the each solution and water control to aid capture of moths entering the trap. Each trap also was baited with a 21 mm polyethylene bottle plug (P/N 25010008A, Kimble Glass, Inc., Vineland, NJ) containing 1 ml of 3-methyl-1-butanol, 98.5+% pure, HPLC grade (Aldrich Chemical Co., Milwaukee, WI). A previous study indicated that the alcohol was essential for attraction of some moth species.

Six treatments (5 molasses solutions plus water control) were set out in 3 randomized complete blocks. The traps were spaced 15 m apart in rows. Two blocks (replicates) were positioned between cotton fields near Trenton, FL, and the third block (replicate) was located in the middle of a circular (67 ha) field of peanuts near Alachua, FL. The test was set out 20 July and terminated 12 August. Insects were collected every 1-2 days, except weekends; and the treatments were re-randomized. After each collection, the test solutions were topped off as needed with the original molasses mixture to maintain the prescribed 250 ml level. The trapped insects were bagged and returned to the laboratory for identification and counting.

Results: A total of 559 fall armyworm moths were captured in all traps over the test period. Of this total, 65.8% were females and 34.2% were males (paired t-test, $t=-9.821$, $P=<0.001$,) (Fig. 1). Of the females captured, 62.1% had mated at least once as indicated by the presence of a spermatophore in the bursa copulatrix.

Only 3.0% of the moths were captured in traps with water only indicating that 3-methyl-1-butanol alone was ineffective as an attractant for fall armyworm.

The 2.5% molasses solution was the most effective dosage for capturing both sexes of fall armyworm (Fig. 1). There was a significant drop off in the number of moths caught in molasses-baited traps at concentrations lower and higher than 2.5%. However, the proportion of females to males captured was about the same, 65:35, regardless of the percentage of molasses used.

These results are very encouraging and could lead to a reliable system for trapping females of the species. A lure that consistently attracts females would be very useful in monitoring pest populations and for direct control using attract & kill methods.

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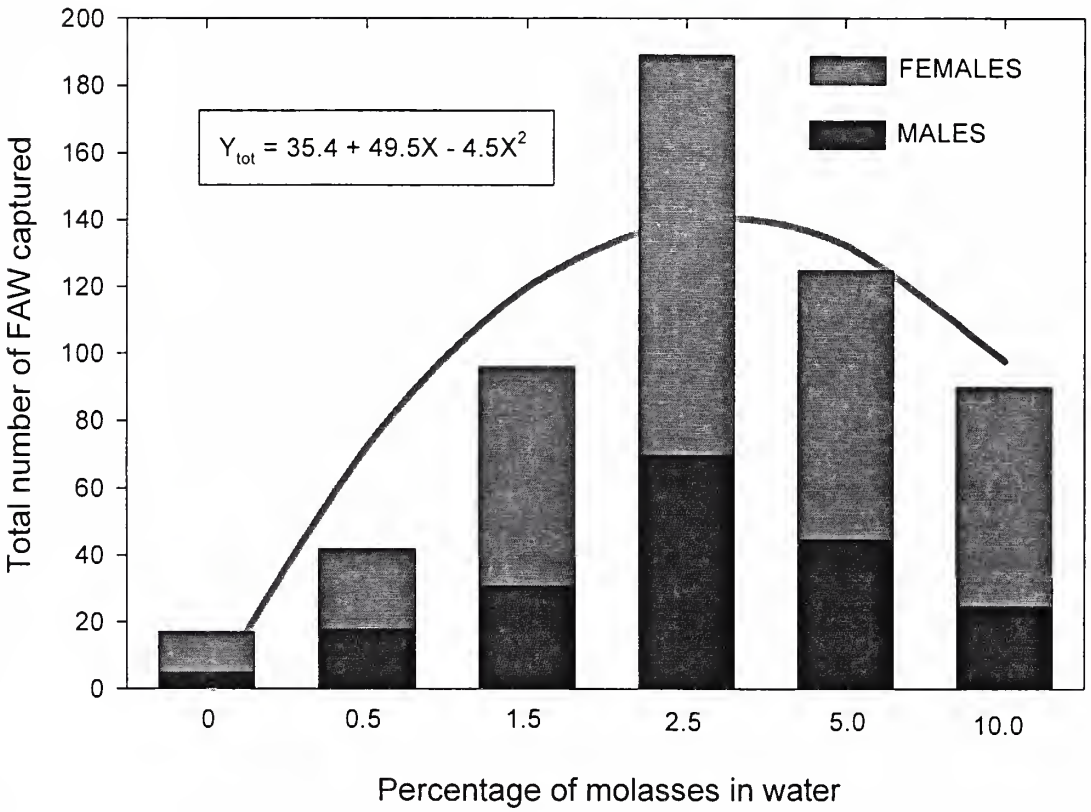
Fig. 1. CAPTURE OF FALL ARMYWORM MOTHS IN DOME TRAPS BAITED WITH MOLASSES AND 3-METHYL-1-BUTANOL



CONTROL OF RATE OF EMISSION OF PHENYLACETALDEHYDE FROM PLASTIC CAPS FOR TRAPPING FEMALE MOTHS

E.R. Mitchell, R.A. Meagher and M.S. Mayer

Objectives: Phenylacetaldehyde (PAA) has been shown to trap both female and male moths of several noctuid pests, but its effectiveness is difficult to assess for several reasons. One is that PAA polymerizes and another is that it is highly volatile and its emission is difficult to control. The studies reported here were to develop a means to control the emission of this compound so that a dose-response analysis of effectiveness could be obtained.

Methods: Two hundred or 500 µl of neat PAA was pipetted into polyethylene stoppers (Part no. RS5212-5, to fit stopper vials #6097SL-3, Kimble Glass, Inc., Vineland, NJ) through the opening accessible by removing the lid. Openings of three sizes, 2.5, 4, and 6 mm were made through the lids and a 2.5 cm^2 membrane (Norton Seal View, A1069796, Norton Performance Plastics Corp., PO Box 3660, Akron, OH 44309-3660) was snapped into place between the opening and the lid. Control stoppers were prepared with an intact lid. Samples of the airborne volatiles were aspirated at various time intervals from time = 0 to 2 weeks and the emission rate of PAA measured (ng/stopper/hr) by gas chromatographic procedures. Several variations on the above concept were tested.

Results: Measures of emission of PAA at t = 0 showed emission immediately after preparation (Table 1). There was no correlation between the emission rate and the size of the hole in the lid. With 200 µl fills, the emission rose to a peak and began diminishing after 2 weeks. The emission data suggest that the PAA is emitted from around the lid. Research is continuing toward development of an effective controlled release system for PAA, both in house and with a commercial firm.

Table 1. Emission of PAA from plastic stoppers, some without holes in lid and some with designated diameters.

Experimental Condition	Avg. Emission rate (ng / stopper / hr)
Experiment 1 (200 µl PAA; lid hole diam.; 2.5 cm^2 membranes)	
none	199.0
4.0 mm	607.2
6.0 mm	759.7
Experiment 2 (200 µl PAA; lid hole diam.)	
none	255.4
2.5 mm	485.0 ± 22.3[1/]
4.0 mm	548.2
6.0 mm	688.3

1/ Average of 4 stoppers ± standard error of mean.

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OVIPOSITION RESPONSE OF *COTESIA PLUTELLAE* (HYMENOPTERA :BRACONIAE) TO STERILE AND NORMAL DIAMONDBACK MOTH (LEPIDOPTERA: PLUTELLIDAE) LARVAE AND SPATIAL DISPERSION OF LARVAE ON COLLARD PLANTS.

J.S. Okine[1], E.R. Mitchell, J. Carpenter[2] and G.Y. Hu[1]

Objective: Determine the feasibility of using sterile diamondback moth (DBM) larvae as host for *Cotesia plutellae*, released argumentatively under field conditions.

Methods: Five individually potted collard plants (*Brassica oleracea* var. *acephala*) were each infested with either 20 second instar normal or sterile DBM (*Plutella xylostella*) larvae and randomly set in an outdoor screen cage (2.7 x 2.3 m). After acclimatization, 25 mating pairs of *Cotesia plutellae* (3 d old) were introduced into the cage for 48 h. Immediately thereafter, the larvae were collected and brought into the laboratory and allowed to complete development on collard foliage. Foliage consumption was estimated.

To determine fitness, three F1 mated *C. plutellae* females from sterile and normal larvae were allowed to oviposit for 24 h into 20 late second instar DBM larvae. The proportion of larvae parasitized was obtained by counting the number of parasitoid pupae that developed.

To evaluate the potential of sterile and normal DBM larvae to migrate onto cabbage from collard plants, the local movement of DBM larvae from hatch to pupation was measured under laboratory and field conditions.

Results: There was no significant difference in parasitism (F=0.17, df=1,9, P=0.6885) between F_1 sterile larvae and normal larvae. The proportion of larvae that survived did not differ significantly between the two groups for the 48 hr sting period (F=0.24, df=1,9, P=0.6355). Larval consumption of collard leaf did not differ between the two sets of larvae (F=0.16, df=1,49, P=0.9971). The developmental period from hatch to pupation was 15 d for both types of larvae. Pre-eclosion time for *C. plutellae* pupae was 4 days, and percent adult emergence was 80.5 $\pm$ 8.8% for sterile larvae and 79.2 $\pm$ 6.3% for normal larvae (F=0.28, df=1,9, P=0.6108). Percent parasitism from F_1 *C. plutellae* was not significantly different between sterile and normal larvae (F=0.11, df=1,19, P=0.7411). Under laboratory conditions the distance moved was not significantly different between sterile and normal larvae (F=0.16, df=1,9, F=0.9971). Under field conditions, the distance moved by sterile and normal larvae was 9 $\pm$ 1.3 cm and 13 $\pm$ 1.6 cm, respectively. These results demonstrate that percent parasitism of diamondback moth by *C. plutellae* is not affected by sterilization of the larvae. Thus, use of sterile diamondback larvae in a biological control program would provide the following benefits: (1) a host for augmentative release of parasitoids, and (2) reduced pest populations through the F_1 sterility principle.

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DEVELOPMENTS OF METHODS FOR PROCESSING LARGE QUANTITIES OF DIAMONDBACK (*PLUTELLAE XYLOSTELLA*) MOTH EGGS FOR FIELD RELEASE

J.S. Okine[1], E.R. Mitchell and G.Y. Hu[1]

Objective: To develop a processing method for obtaining diamondback moth (DBM) eggs for seeding crops using a spraying machine.

Methods: We first determined the best substrate for egg acquisition: Corn starch jelly, corn starch suspension and Egg Beaters™ , all mixed with a water extract of collard leaves were smeared on 4 X 15 cm organdy cloth. Strips of the treated cloth were hung in a cage containing several hundred 3-4 d old diamondback moths for 24 hours, after which the clothes were removed and the number of eggs on each counted under a dissecting microscope. Thereafter, we determined the most efficient way to liberate the eggs from the substrates without affecting viability. The substrates were treated in the following manner: 1) Substrates were soaked for 3 minutes in a 1.7% bleach in water solution; 2) substrates were then rinsed in water; 3) substrates were soaked again for 3 minutes in a neutralizing solution; and 4) substrates were washed again to detach eggs.

The eggs liberated per substrate were removed with a dropper and put on moist organdy cloth. They were counted under a dissecting microscope. The number of eggs liberated per substrate was analyzed statistically. Viability of the eggs were ascertained by putting 10 eggs on collard plants and counting the number of larvae that hatched in 6 days.

Five hundred eggs obtained from the processing method were put in a mixture of Biocarrier™ jelly and water in a ratio of 1:8. This was poured into the container of a Smucker's Sprayer™ and the pressure set at 15, 18 and 20 psi respectively. The eggs were sprayed on 3 potted collard plants assembled close together to provide a large volume of foliage.

Results: There was no significant difference in mean number of eggs laid on each substrate. There also was no significant difference between the number of eggs liberated from the corn starch jelly and the corn starch suspension-treated substrate. The numbers of eggs liberated from the Egg Beaters-treated substrate was significantly lower than those liberated from the corn starch jelly and suspension.

The viability of the eggs liberated was >90% for all suspensions tested.

Only a small number of eggs stuck to the foliage of the potted collard plants regardless of the air pressure used in the sprayer.

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OVARIAN-SPECIFIC EXPRESSION OF THE THE YOLK PROTEIN GENE IN *ANASTREPHA SUSPENSA*

A.M. Handler

Objective: To isolate a cDNA clone of the Caribbean fruit fly, *Anastrepha suspensa*, 48 kDa yolk protein gene to perform comparative molecular sequence analysis and perform developmental assays for gene expression.

Methods: A lambda gt11 cDNA expression library was constructed from poly A^+ RNA from *A. suspensa* vitellogenic ovaries using standard procedures. The expression library was screened with yolk polypeptide polyclonal antibody, with positively reacting bacteriophage re-screened with *D. melanogaster* yolk protein 1 radiolabelled probe. The DNA from positive bacteriophage was purified and subcloned into pUC19. The pUCAsYP plasmid subclones were sequenced on both strands with sequence alignments performed using GeneWorks 2.3 software (Oxford Molecular Group), and database comparisons using BLAST. Developmental northern RNA hybridization analyses were made by extraction of RNA from female and male adults and dissected tissues of various ages, and whole insects and isolated abdomens injected with 20-hydroxyecdysone or a juvenile hormone analogue, methoprene. RNA blots were hybridized to radiolabelled pUCAsYP probe.

Results: A partial 1.4 kb cDNA clone for the 48 kDa yolk polypeptide gene from *A. suspensa* was isolated from an ovarian cDNA expression library using *A. suspensa* yolk protein antibody and the *D. melanogaster* yolk protein 1 gene as probes. The sequenced yolk protein cDNA has greatest homology to the yolk protein genes from *Ceratitis capitata*, *D. melanogaster*, and *Calliphora erythrocephala*, and similar to these genes, shares amino acid sequence domains with those from lipases. RNA hybridization studies indicated that the yolk protein gene expression is completely female-specific and limited to the ovaries, without apparent regulation by 20-hydroxyecdysone or juvenile hormone. This is in contrast to an earlier study which suggested, based on immunological probes, that a very low level of yolk protein synthesis occurred in fat body and was not sex-specific. This is a unique finding in that yolk protein synthesis is generally limited to the fat body in most insects, or the ovaries in addition to the fat body in higher dipterans, and is under hormonal regulation. Based on biochemical tests, only one other insect, the stable fly, has yolk protein synthesis limited to the ovaries. This data has relevance to prospective biological control strategies that would target specific tissues or physiological processes important to insect reproduction.

TRANSFORMATION OF *DROSOPHILA MELANOGASTER* WITH THE *TRICHOPLUSIA NI* TRANSPOSON VECTOR, *PIGGYBAC*, MARKED WITH THE GREEN FLUORESCENT PROTEIN

A.M. Handler and R.A. Harrell II

Objective: To test the ability of the *Trichoplusia ni piggyBac* transposon vector to mediate germline transformation in dipteran species, and test the function of a new green fluorescent protein marker (GFP) construct.

Methods: The *T. ni piggyBac* transposon vector was previously shown to mediate efficient gene transfer in the Mediterranean fruit fly, *Certaitis capitata,* using the medfly *white*$^+$ marker. To determine if *piggyBac* could function similarly in other distantly related dipterans, germline transformation was attempted in *D. melanogaster.* Transformation was tested first using the *Drosophila white*$^+$ marker with an unmodified self-regulated transposase helper, and secondly with a heat shock promoter regulated (*hsp70*) helper. In another experiment a new marker having GFP under polyubiquitin-nuclear localizing sequence regulation, in addition to the *white*$^+$ marker, was tested. A mixture of vector and helper plasmids, at concentrations of 600 μg/ml and 400 μg/ml, respectively, was injected into preblastoderm embryos for each experiment. Standard injection and rearing procedures were used, with G0 adults (arising from the injected embryos) backcrossed to flies from the white eye host strain, *w*[m]. First generation (G1) offspring were screened for expression of eye pigmentation under visible light, and for green fluorescent protein expression under long wavelength ultraviolet light. Putative transformants were backcrossed to *w*[m] flies with offspring inbred to create homozygous lines. Molecular verification of *piggyBac* vector integrations into chromosomal DNA was achieved by Southern DNA hybridization analysis and sequencing of insertion sites after isolation by inverse PCR.

Results: Autonomous *piggyBac* function was shown in *D. melanogaster* by use of a self-regulated transposase helper and a vector marked with a mini-*white* gene. A transformation frequency of 1-3% per fertile G0 was obtained, similar to that previously achieved in the Mediterranean fruit fly. Use of a transposase helper under *hsp70* promoter regulation increased the transformation frequency by more than eight-fold (26%), yielding a rate comparable to other vector systems utilizing a heat-shock regulated helper. Addition of a GFP marker gene under polyubiquitin-nuclear localizing sequence regulation to the vector also resulted in transformants, but notably, most of the 70 G1 transformants (from seven G0 lines) visibly expressed only the GFP, with extremely little or no eye pigmentation observable. Chromosomal position effect suppression appears to have a significantly greater influence on the phenotypic expression of *white* than GFP, which is encouraging for the further use of GFP as a gene transfer marker in other insect systems. Transformation in two distantly related dipteran species with the *piggyBac* vector is also supportive for its potential use in other dipterans, and perhaps insects in general.

CRIS 6615-22000-013-00D

DIAMONDBACK MOTHS PERCEIVE DIFFERENCES BETWEEN ATTRACTIVE SEX PHEROMONE MIXTURES THAT ARE NOT MEASURABLE BY ANALYSIS OF LURE EMISSIONS

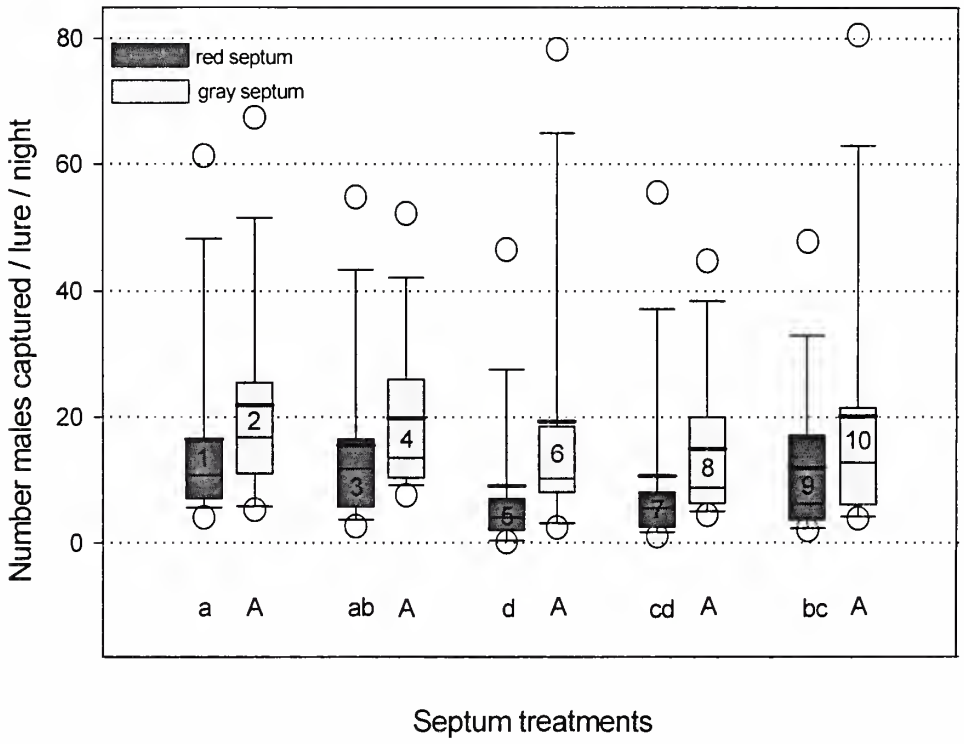
M.S. Mayer and E.R. Mitchell

Objective: To measure the emission rates of active components from two lure substrates. These measures provide crucial emission rate data which can be used to determine how close a synthetic mixture mimics the innate sex signal; and secondly to optimize captures for surveys and as a measure of local populations.

Methods: Five different mixtures totaling 100 µg of Z11-16:Al, Z11-16:Ac, Z11-16:OH, and Z11-14:Ac were assayed on red and gray halobutyl rubber septa in Pherocon 1C traps. An untreated septum was used as a control. The experimental design of the trap assay consisted of 3 blocks of 11 locations each along the margins of different cabbage fields. The number of males trapped was counted on a regular basis and baits were re-randomized in different positions. Variance was stabilized by square root transforms of trap counts and analyzed by split-plot analysis of variance. Samples of airborne emissions from each septum were obtained for each treatment for six time intervals over a 2-week period. Septa were extracted after mincing in a 1 : 1 mixture of hexane and methylene chloride containing an internal standard of 100 µg of n-tetradecane. We recovered 98.3 percent of the extraction standard. Samples were analyzed by conventional gas chromatographic methods. The emission rates of each compound from the septa were examined by regression analysis to determine whether or not different compounds deviated from linearity over time.

Results: The average numbers of males captured with gray septa (2, 4, 6, 8, 10) was greater than the average captured with red septa (1, 3, 5, 7, 9) across all treatments (Fig. 1). There were no statistical differences in moth captures among any of the pheromone blends on gray septa (2, 4, 6, 8, 10), but among the red septa the 70:30 blend captured significantly more males than the 50:50 (5, 7) blends. Treatment 1 (70:30 blend) also was different from treatment 9 (67:23 blend) (Fig. 1). The overall average emission rate and standard error of Z11-16:Al, Z11-16:Ac and Z11-16:OH from all ten treatments was 2.9 ± 0.7, 1.5 ± 0.2, and 60.7 ± 13.9 ng / lure / hr, respectively. Extractive measures of the components revealed an average loss of 62.0 % of Z11-16:Al from the five treatments on gray septa and 59.8 % from five treatments on red septa which occurred under seal and refrigeration in about 50 days. The combined results from trap assays and emission data impact development of better lures for diamondback moth and findings on attraction attributed to differences in biotypes.

Fig. 1. Tukey plot of diamondback moth captures in Pherocon 1C sticky traps baited with 5 different blends of pheromone, each on red or gray septa. Heavy line in box is mean.



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ECOLOGY AND BEHAVIOR OF TEPHRITID FRUIT FLY PARASITOIDS IN MEXICO AND FLORIDA

J. Sivinski and M. Aluja[1]

Objective: Biological control sometimes has a major affect on pest fruit fly populations, but in other instances parasitoids either fail to become established or do not flourish and are only rarely recovered. One reason for the failures may be that the wrong parasitoids for the local conditions are being used in control efforts. Parasitoids, including those that attack tephritids, are often specialized. That is, they are active in certain trees, locations, seasons, and times of day. Information on when, where, and how natural enemies hunt for pest fruit flies may help predict which species should be introduced or periodically released in large numbers (augmented) in particular areas.

Methods: In Veracruz State, Mexico the spatial and temporal distributions of braconid, eulophid, eurytomid, eucoilid, and diapriid parasitoids attacking five species of *Anastrepha* fruit flies have been studied for five years. Samples have been taken to determine altitudinal and regional patterns of abundance and laboratory tests have been used to examine the propensity of parasitoids to enter diapause (a seasonal pause in development that allows an insect to avoid unfavorable conditions). In Florida area wide samples of three parasitoid species were collected at different latitudes and the environmental correlates of their distributions identified.

Results: Altitudinal transects sampling parasitoids in guava fruits have been completed and find that various parasitoids are most abundant at different altitudes. These distributions are consistent with previous latitudinal transects and also support findings that host fruit density is an important factor in the survival and increase of certain parasitoids. The last point may be particularly important. Since most parasitoids are not as capable of dispersal as their fruit fly hosts, a local variety of trees, fruiting at different times, may provide the best conditions for parasitoids to become established and increase in numbers. A long-experiment where native fruit tree diversity will be re-established near agricultural areas is underway. A nursery has been founded and several hundreds of seedlings are being grown. These trees will harbor nonpest fruit flies that are attacked by the same parasitoids as the pest species. By providing alternative hosts it is hoped that parasitism rates will increase and infestation rates decrease. Diapause is another means whereby parasitoids could persist over times of low host abundance. Further evidence for wide-spread diapause has been obtained, including the first data that diapause may last more that 1 year. Such a finding emphasizes the importance of long term studies that take into account yearly changes in environmental conditions and which are likely to discover lengthy phenomena such as diapause.

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BIOLOGICAL CONTROL OF MEDFLY IN GUATEMALA

J. Sivinski, T. Holler[1], and F. Jeronimo[2]

Objectives: The Mediterranean fruit fly is abundant in Central America and threatens to move northward in the Mexico and ultimately the United States. It is prevented from doing so by a sterile fly and pesticide barrier maintained on the Mexican-Guatemalan border by the international organization MOSACMED. However, in recent years this barrier has become increasingly permeable and new techniques are being sought to both improve it and make it more environmentally benign. Biological control has two possible roles in the region: 1) Large numbers of mass-reared parasitoids can be combined with sterile fly releases, and 2) new parasitoids can be introduced that will result in fewer adult flies and so contribute to integrated pest management.

Methods: *Augmented release of parasitoids-* Sterile male medflies and the parasitoid *Diachasmimorpha tryoni* were released by airplane over the mountainous coffee growing regions of northern Guatemala. The parasitoids were chilled, placed in paper bags and dropped from an altitude of ~100m. Releases were done in two locations and at three different densities. Controls consisted of untreated plots and those over which sterile flies alone were released. In addition the feasibility of using a automated cooling box and auger release machine to replace the paper bags was examined. *Parasitoid introduction-* At present parasitism of the medfly in Guatemala is low and sporadic. Explorations for more effective parasitoids for use both in establishment attempts and augmented releases are ongoing in Mexico and Kenya.

The later collections are part of a collaborative effort with the Universities of Hawaii, Florida and Texas A&M. Candidate parasitoids are colonized at USDA-APHIS facilities in Guatemala where laboratory and field cage studies are undertaken to determine host range, ability to develop in irradiated hosts, and propensity to compete parasitoids already present.

Results: *Augmented releases-* There was a positive correlation between the numbers of parasitoids released and the percent parasitism of medflies. The highest parasitism levels were reached at rates in excess of what previous studies would have suggested to be necessary. It may be that *D. tryoni* is not the best suited parasitoid for use at the relatively low host density typical of late season coffee in Guatemala. Parasitoids ejected from the cooling box and auger release machine suffered no more mortality than, and were as active as, those parasitoids released in paper bags. *Parasitoid introduction-* Five parasitoids are in colony. These include the first of the new African species, *Psyttalia humilis*, and the highly effective egg parasitoid *Fopius arisanus.* Field cage studies with *Diachasmimorpha krausii* have shown it to survive and attack medfly under Guatemalan conditions. it also appears to have a relatively narrow host range in that it thrives on medfly but rarely develops in *Anastrepha* spp. Considerable progress has been made in rearing the pupal parasitoid *Coptera haywardii.* In addition, a new pupal parasitoid, an unusual species of Eurytomidae, has been discovered in Mexico where initial colonization is taking place.

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BIOLOGICALLY BASED PEST MANAGEMENT THROUGH ARTIFICIAL REARING OF NATURAL ENEMIES AND MANIPULATION OF HOST PLANT RESISTANCE

S.M. Ferkovich, J.A. Morales-Ramos[1], M.G. Rojas[1], H. Oberlander, J.E. Carpenter[2] and P. Greany

Objective: To investigate the potential of providing requisite host factors or their products in sub optimal diets for parasitoids or predators through the use of insect cell lines and/or their products.

Methods: When reared on an artificial diet (DI diet), wasps were smaller, took longer to develop, and had reduced fecundity compared with wasps reared on the natural host. Grace's culture medium was conditioned for 24 h with IPL-LdFB, a cell line derived from fat body of the gypsy moth, *Lymantria dispar* (Linnaeus) and Sf9, a cell line derived from ovaries of the fall armyworm, *Spodoptera frugiperda*. The cell-conditioned medium from each cell line was then used to supplement the original DI diet. Fresh supplemented diet was presented to the larvae on day four. The cell line-supplemented diets were also chemically analyzed for protein, lipids, vitamins, minerals, and supplemented with additional nutrients where necessary. Aliquots of each diet were encapsulated in paraffin domes and newly hatched larvae of *D. introita* were placed on each diet (one larva/dome) and allowed to develop to the adult stage.

Results: Providing fresh diet on day four when the larvae were in the third instar did not improve parasitoid production. Compared with the DI diet, only the Sf9 cell line-supplemented diet (Sf9CellCond) had a positive effect on the parasitoid's growth, increasing the size of male parasitoids. The parasitoids, however, took longer to develop to the adult stage. Neither of the cell lines significantly enhanced the average weight of female parasitoids, nor shortened developmental time, nor increased % cocoon production and % adult emergence. Providing additional nutrients (amino acids, vitamins, cations and anions, fatty acids and milk/egg protein) to both diets (based on chemical analyses of the cell line-supplemented diets) also enhanced the average weight of the females on the Sf9CellCond diet and males and females on the IPL-CellCond diet. The additions, however, but did not improve the other growth qualities measured.

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ARTIFICIAL REARING OF THE GENERALIST PREDATOR *PODISUS MACULIVENTRIS*

P. Greany, S. O'Keefe[1] , and I. Baez[2]

Objective: To develop a low-cost artificial medium that supports growth, development, and optimal fecundity of the predatory hemipteran, *Podisus maculiventris*, to enable mass production in support of its use in augmentative biological control.

Methods: An artificial diet developed earlier for an ectoparasitoid (U.S. Patent 5,799,607) was modified in composition and the means for storing the medium before use was altered.

Results: We have been able to increase the fecundity of Pmac females reared on the diet from about 50 eggs per female to about 250 eggs per female over the past year. This was achieved principally by storing the diet in the freezer prior to use, rather than in the refrigerator, and by addition of an antioxidant, tertiary butylhydroquinone (TBHQ). Chemical analyses have shown that polyunsaturated fatty acids can be oxidized during refrigeration, but this has not been shown to be the cause of reduced fecundity. Diet-reared Pmac females that are provided *Galleria mellonella* larvae as adults produced nearly as many (ca. 500) eggs as females that were fed *G. mellonella* larvae throughout their lies, suggesting that the diet-reared females were reproductively competent, and that reduced fecundity is a reflection of a less-that-optimal diet. Chemical analyses are now being performed on the effect of storage conditions on dietary vitamin E levels. We also are going to perform tests on the effect of replacing the diet on a daily basis, rather than 3 times per week, to determine the best possible outcome from the existing diet. Supplementation of the diet with additional cholesterol, which could be a limiting factor in egg production, also is planned.

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CHEMISTRY

CRIS - 6615-22000-012-00D--Chemistry and Biochemistry of Insect Behavior, Physiology, and Ecology

CRIS 6615-22000-012-00D

ISOLATION AND IDENTIFICATION OF PLANT VOLATILE ELICITORS FROM *MANDUCA sexta* ORAL SECRETIONS

H.T. Alborn, M.M. Brennan and J.H. Tumlinson

Objective: To isolate and identify substances in the oral secretions of *Manduca sexta* caterpillars that induces plants to biosynthesize and release volatile compounds.

Methods: *Manduca sexta* caterpillars were collected from tobacco fields in Alachua County, FL. Oral secretion was obtained by squeezing the caterpillars and collecting the oral secretion in a vial. Oral secretion is stored at -70EC. Crude oral secretion is centrifuged at 14,000*g* for 10 min to remove solids and the supernatant is then filtered through 0.45µm and 0.22 µm sterilizing membranes. Active material is extracted from an acidified aqueous filtered supernatant with methylene chloride. Separation and purification of the active compounds is achieved by reverse phase HPLC on a C18 column. A bioassay which consists of gas chromatographic analysis of the volatile compounds emitted by corn seedlings is used to monitor fractionation of the oral secretion. An amount of each fraction is added to 500 µl of 50 mM, pH 8, phosphate buffer. A 9- to 10-day-old corn seedling is cut off above the root with a razor blade and the cut end immersed in the buffer solution in a 1 ml glass vial. The seedling is allowed to draw up the solution over an 17-hr. period . Then one seedling for each treatment is put in in a glass volatile collection apparatus (18 cm long, 3.5 cm id) under artificial light. Purified, humidified air is drawn through the chamber and then through a polymeric adsorbent (Super Q) at 500 ml/min for 2 hr. The adsorbent is extracted with 170 µl of methylene chloride and the extract analyzed by capillary GC.

Results: The crude oral secretion of *M. sexta* caterpillars induces corn seedlings to produce and release a similar blend of volatile compounds, but in much smaller quantities (about 10%), than the oral secretion of *Spodoptera exigua* caterpillars, or a solution of its active ingredient, volicitin. In contrast to *S. exigua* oral secretion, where volicitin showed a very dominating biological activity, several components of *M. sexta* oral secretion induce the release of plant volatiles by corn seedlings. The active components can be extracted from an acidified aqueous solution of the oral secretion into methylene chloride, which indicates that they are similar to volicitin in that they have both lipid character and acidic functional groups. Two active components have been isolated, identified and synthesized.

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Mode of Action of Pheromonotropic Neuropeptides in Regulation of Pheromone Biosynthesis in *Mamestra brassicae*

A. Fonagy[1], P.E.A. Teal and J.A. Meredith.

Objectives: To determine the mode of action of pheromonotropic neuropeptides in induction of pheromone biosynthesis in *Mamestra brassica*.

Methods: Studies of the cellular mode of action of neurohormonally controlled pheromone production were conducted using synthetic PBAN along with various pharmacochemicals (stimulators or inhibitors) under *in vivo* and *in vitro* (isolated pheromone glands were incubated in media containing respective agents, then extracted) conditions. We measured changes and fluctuation of cAMP in gland cells using the Amersham "Cyclic AMP (3H) assay system" following various *in vivo* and *in vitro* treatments.

Results: Under *in vivo* conditions, following the injection of PBAN, the level of cAMP reached it's maximum at 15 min, while maximum pheromone production required ca. 60-90 min. Under *in vitro* conditions a significant cAMP peak was detected at 2-3 min, followed by other two fluctuating minor peaks. The pheromone production pattern was, however, similar to the *in vivo* results. PBAN induced cAMP formation in a dose-dependent manner as did the Ca ionophore and Ionomycin. A similar dose response was found with Forskoline (adenylate cyclase activator). The use of $LaCl_3$ inhibited Ca++ influx via plasma membrane and when incubated with PBAN no increase in cAMP or pheromone was detected. We also found a low level of cAMP when glands were co-incubated with trifluoroperazine (inhibitor of Ca++ Calmodulin complex) and PBAN. The results suggest that stimulation of pheromone production (Z11-16Ac) is modulated by cAMP with the invovement of Ca++/Calmoduline system.

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THE BIOSYNTHESIS OF AN INSECT ELICITOR OF PLANT VOLATILES

P.W. Paré, H.T. Alborn and J.H. Tumlinson

Objective: To determine the biogenetic origin of *N*-(17-hydroxylinolenoyl)-L-glutamine, an elicitor of plant volatiles, named volicitin and isolated from beet armyworm caterpillars.

Methods: Corn seedling (*Zea mays* L., variation LG11 sweet corn) were grown for twelve days under artificial lights in glass chambers in which synthetic premixed air containing 1800 µM/liter CO_2 (^{13}C 99.9%) flowed continuously. Volatile compounds were collected from damaged leaves and ^{13}C content for the linolenic acid derived volatiles were measured by mass spectrometry to monitor incorporation levels. Subsequent to the feeding experiments (see below) the remaining portions of the leaves of each plant were extracted and analyzed to determine the percentage of incorporation of the label in linolenic acid and glutamine; identification of plant metabolites was confirmed by GC/MS comparison with synthetic standards. Beet armyworms (*Spodoptera exigua* Hübner) were reared on artificial pinto diet and transferred to feed on corn seedlings at least 48 hours before labeling experiment. The oral secretion from five larvae was collected and 5 µL of aqueous *N*-palmitoleoyl-L-glutamine solution (1 µg/µL) was added as an internal standard. Regurgitant was fractioned by HPLC with an acetonitrile-water gradient. Peaks eluting from this column (Fig. 1) were collected, lyophilized and treated with methanol and acetic anhydride , and the derivatized products were analyzed by GC-MS.

Results: To assess the rate of synthesis and the source of the chemical components used by beet armyworm to assemble volicitin, caterpillars were fed on corn seedlings that were labeled uniformly with ^{13}C. Analysis of compounds purified from oral secretions of insects fed on unlabeled and labeled seedlings revealed the consistent presence of nine compounds (Fig. 1). In addition to volicitin, beet armyworm oral secretions contain free 17-hydroxylinolenic acid, *N*-linolenoyl-L-glutamine and free linolenic acid, and an analogous series of compounds with a linoleic acid backbone. The mass spectral data for the methyl ester of linolenic acid, as well as the fatty acid portions from the other beet armyworm components demonstrated extensive incorporation of ^{13}C. Within 6 hours, the acids in beet armyworm oral secretions contained a level of ^{13}C-labeling comparable to what is found in the labeled seedlings. For the fatty acids analyzed from the caterpillars, as well as the labeled corn seedlings that they fed on, the pattern of ^{13}C incorporation followed a gaussian distribution with m/z = 305 representing slightly more than half of the carbon atoms containing the ^{13}C label. This indicates that the fatty acid portion of volicitin and the other conjugated compounds in the insect oral secretion are obtained directly from the plant. In contrast the glutamine incorporates little ^{13}C relative to the glutamine from the plant indicating that the plant is not catalyzing the coupling of glutamine to the fatty acids. These biochemical data demonstrate that the plant supplies linolenic acid, which is required for growth and development of beet armyworms, and also provides this fatty acyl chain for the synthesis of volicitin, the modified elicitor of plant volatiles that is central to signaling between plants and natural enemies of the caterpillars that attack them.

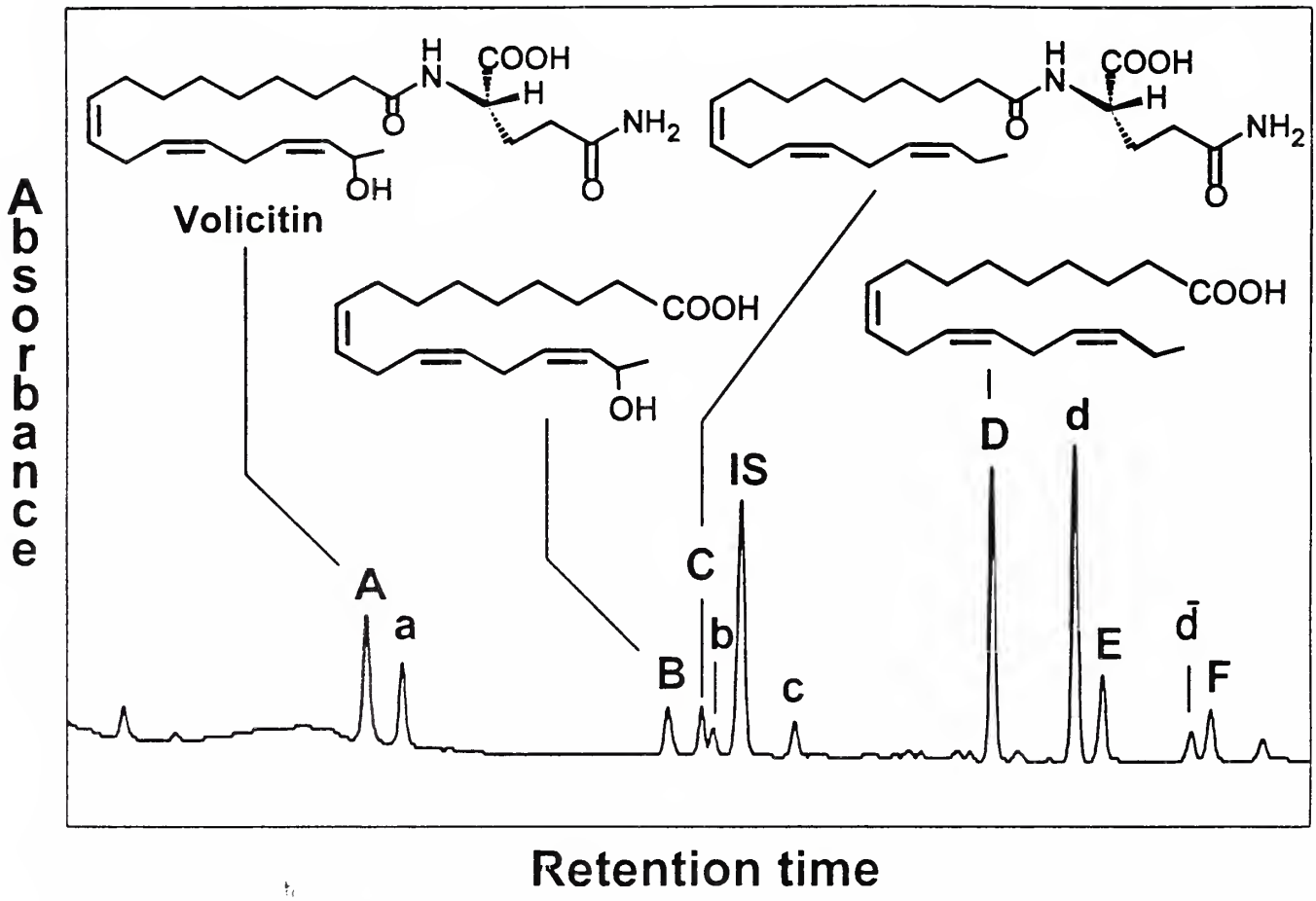

Fig. 1. HPLC profile of volicitin (**A**) and other fatty acid derivatives detected in the oral secretions of beet armyworms at a wavelength of 200 nm. Compounds and approximate retention times include: (**A**) *N*-(17-hydroxylinolenoyl)-L-glutamine (7.5 min), (**a**) *N*-(17-hydroxylinoleoyl)-L-glutamine (8.0 min), (**B**) 17-hydroxy linolenic acid (11.1 min), (**C**) *N*-linolenoyl-L-glutamine (11.9 min), (**b**) 17-hydroxy linoleic acid (12 min), (**IS**) *N*-palmitoleoyl-L-glutamine (internal standard) (12.3 min), (**c**) *N*-linoleoyl-L-glutamine (12.6 min), (**D**) linolenic acid (15.2 min), (**d**) linoleic acid (16.2 min), (**E**) unknown (16.6 min), (**d̄**) oleic acid (17.1 min), and (**F**) impurity (17.8 min).

CRIS 6615-22000-012-00D

Induction of Pheromone Production in Females of *Heliothis virescens* by Topical Application of an Amphiphylic Pseudotetrapeptide Analogs of Pheromone Biosynthesis Activating Neuropeptide.

P.E.A. Teal, R.J. Nachman[1] and J.A. Meredith.

Objectives: To design and develop synthetic analogues of insect neuropeptides that penetrate the insect cuticle and maintain bioactivity.

Methods: Pseudotetrapeptide analogs of the C-terminal active core (FSPRLamide) of pheromone biosynthesis activating neuropeptide (PBAN) were synthesized by addition of aliphatic fatty acids to the aminoterminal phenylalanine. Pheromonotropic activity of the analogs were assessed in injection bioassays in which females of *Heliothis virescens* were injected with different doses of the analog or PBAN. Females were incubated for 1h after injection and then the sex pheromone glands were excised and extracted in hexane containing internal standards. The extracts were then analyzed by capillary gas chromatography to determine the amount of pheromone present. Topical application studies were conducted by applying various doses of the pseudopeptides analog or PBAN to the descaled abdomen in water. After incubation the sex pheromone glands were excised, extracted and the extracts analyzed for the amount of pheromone present as above.

Results: The pseudopeptide analogs were capable of stimulating pheromone production when injected into females of *H. virescens*. However, not all of the analogs induced production of pheromone when applied topically to the abdomen. The analogs formed by attachment of C5, C8,or C10 fatty acids were equally potent and induced maximal production of pheromone production at doses greater than 50 pmol. The analog formed by attachment of dodecanoate to the peptide required application of 250 pmol for full pheromonotropic activity whereas attachment of palmitic acid to the peptide rendered the peptide inactive when applied topically at concentrations as great as 2 nmol. Cuticle penetration studies indicated that the palmitic acid analog did not penetrate the insect cuticle.

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Endogenous Regulation of Mating and pheromone Production in Caribbean Fruit Flies.

P.E.A. Teal, Y. Gomez-Simuta, J.A. Meredith and A.T. Proveaux

Objectives: To determine the endogenous factors that regulate the coordination of sexual communication and mating among males of the Caribbean Fruit fly.

Methods: Behavioral studies on the interactions between calling behavior and mating release were studied in cage bioassays. Males were caged with females on the day of emergence and observed for calling behavior and mating during the reproductive period of each day untill all females had mated. Additional studies were conducted by combining virgin females with mated males and observing the time that it took for these males to mate. These data were compared with those obtained when virgin males of the same age were paired with virgin females. Pheromone production by virgin and mated males of the same age was monitored by collecting volatiles released groups of 5 males and analysis of the volatile colections by capillary GC and GC-mass spectroscopy.

Results: Results of behavior studies using virgin males indicated that a small percentage of males began to exhibit calling behavior on day 4 after emergence. This was coupled by release of small, but significant amounts of pheromone. The earliest age at which mating occurred was 5 days after emergence. All males exhibited calling behavior by day 7 but maximal production of pheromone did not occur until the males were 8-days old. All males had mated by day 9. Studies comparing the mating of virgin and mated males 6, 7 or 8-days old indicated that mated males all remated within the first 2h of the reproductive period but that the populations of virgin males required 2 reproductive cycles to complete mating. Analysis of volatile pheromone released by virgin and mated males 6-8-days old indicated that mated males released significantly more pheromone than did their virgin counterparts.

CRIS 6615-22000-012-08R and 6615-22000-012-07T

Development of Female Medfly Attractant Systems for Use in Sterile Fly Eradication Programs

R.R. Heath, N.D. Epsky, B.D. Dueben, J. Hendrichs, B.I. Katsoyannos, L.A. Vásquez, J.P. Ros, A. Zümreoglu, R. Pereira, A. Bakri, S.I. Seewooruthun, H. Camacho, P. Howse, E. Vattuone, F. Jeronimo, P. Montoya, T. Labuschagne, and Y. Rossler

Objective: Develop a female Mediterranean fruit fly trapping system for detecting wild flies during eradication using sterile males. Evaluate the use of the female Medfly lure previously discovered consisting of ammonium acetate, putrescine and trimethylamine in countries having hosts and climates similar to citrus growing areas of the U.S.

Methods: Field experiments were conducted in ten countries under an international research project operated as part of the FAO/IAEA Research Contract Program. The participants consisted of research teams from Argentina, Costa Rica, Greece, Guatemala, Honduras, Mauritius, Mexico, Portugal, South Africa, and Turkey. Field trials were conducted in citrus orchards and in other hosts of the Medfly. Comparisons were made to determine the efficacy of the three component synthetic lure in two types of plastic McPhail type traps. The two McPhail-type traps used were the International Pheromones McPhail Trap^{R} (IPMT) and a Tephri-trap^{R}. Both traps have a funnel at the bottom that permits the flies to enter the trap body. The Tephri trap also contains three holes equally spaced near the trap top. Additional comparisons were made to determine the efficacy of the traps when used with water as the killing agent and when used dry with a small amount of pesticide (DDVP). For comparative purposes standard traps that are used for Medfly detection were included. These were IPMT traps baited with protein bait, NuLure and borax, and Jackson traps baited with the male attractant Trimedlure (TML). Traps were checked twice a week and rotated to the next position and experiments were conducted for 16 weeks in each country.

Results: The results from tests in all countries of wet and dry traps containing the three component synthetic female attractant and IPMT traps baited with NuLure lure is shown in the figure. IPMT wet and dry and the Tephri traps dry captured significantly more flies then the IPMT containing NuLure. The commercial formulation of the three component lure has a field life of 6 - 8 weeks compared to protein baits that should be replaced every week. Traps baited with the synthetic lure capture few non target insects. Protein baits capture 4 to 50 times more non target insects, including beneficial insects, then the synthetic lure. Traps baited with the three component lure captures 5 - 40 times less sterile flies then the male attractant TML baited traps.

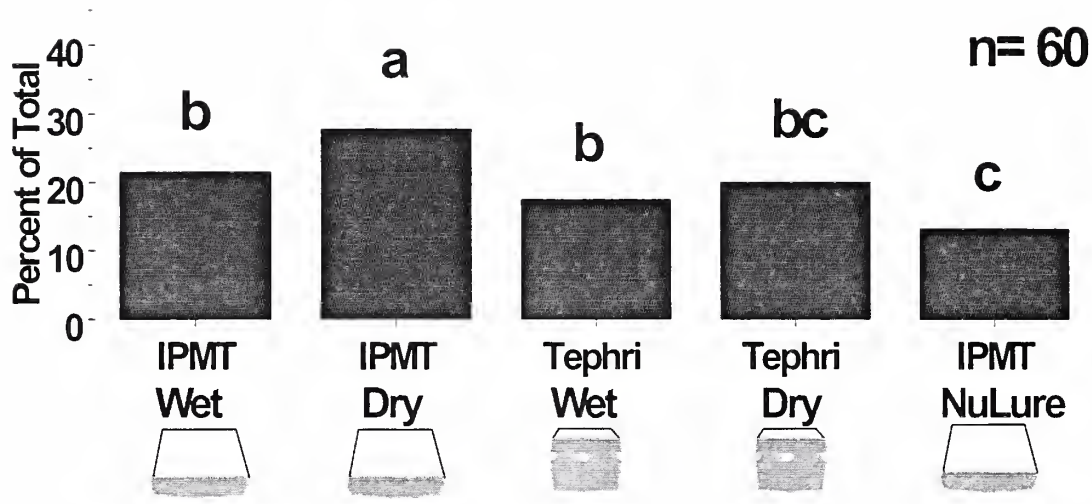

Fig. 1. Comparison of total medfly capture in all countries with wet and dry traps baited with three-component female attractant and IPMTs with NuLure.

Cris # 6615-22000-012-09T

ISOLATION AND IDENTIFICATION OF PLANT VOLATILE ELICITORS FROM *Schistocerca americana* ORAL SECRETIONS

H.T. Alborn, T. Hansen and J.H. Tumlinson

Objective: To isolate and identify the substances in the oral secretions of *Schistocerca americana* that induces plants to biosynthesize and release volatile compounds.

Methods: *Schistocerca americana* nymphs and adults were obtained from Dr. John Capinera, Dept. of Entomology and Nematology, University of Florida and were maintained under a 14:10 LD cycle at 60 % RH and 25 EC and fed corn seedlings. Oral secretion was collected from the grasshoppers by gently squeezing them and drawing the oral secretion into a capillary pipette under a slight vacuum. Oral secretion is stored at -70EC. A bioassay similar to that used to purify volicitin from beet armyworm oral secretion and the active compounds of *Manduca sexta* oral secretions was used to monitor fractionation. Crude oral secretion is centrifuged at 16,000*g* for 30 min to remove solids and the supernatant is then filtered through a 0.22 µm sterilizing membrane. The active compounds are extraction into methylene chloride from a water solution of the oral secretion that has been saturated with a neutral buffer. The methylene chloride extract is evaporated to dryness followed by solid phase extraction on a C_{18} column. Final purification is achieved on a C_{18} reverse phase HPLC column with a buffered mobile phase.

Results: The crude oral secretion of *S. americana* induces corn seedlings to produce and release a similar blend of volatile compounds, but in approximately 6-fold larger quantities, than the oral secretion of *Spodoptera exigua* caterpillars. The active compounds are partially purified by extraction into methylene chloride from a water solution that has been saturated with a neutral buffer. This indicates components with amphoteric characteristics. In contrast, the most active component in *S. exigua* oral secretion, volicitin is acidic. The partially purified components are separated on HPLC using a water/acetonitrile gradient. The HPLC separation revealed a group of several similar components, figure 1, among which only two showed strong activity. Several mg of the active components were collected for identification by repeated injections. These components have been partially identified using NMR, and mass spectrometric techniques.

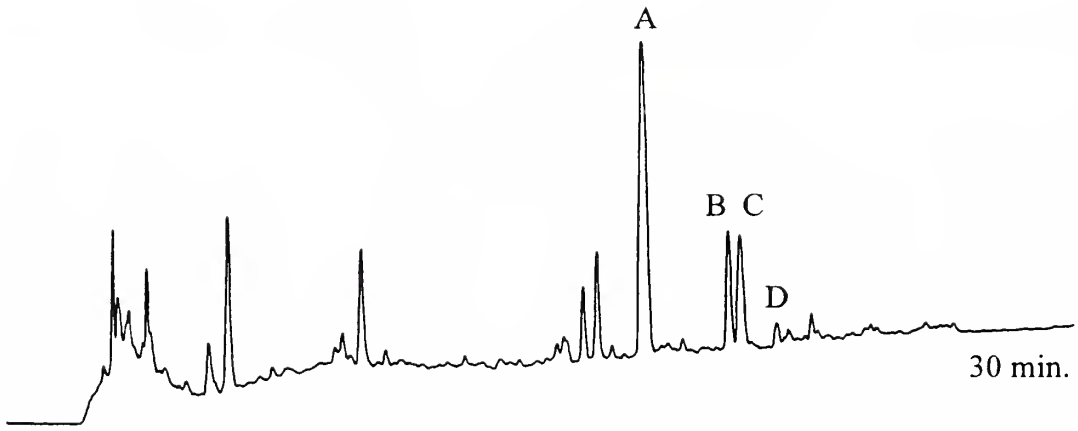

Figure 1. HPLC trace of partially purified *S. americana* oral secretion. Peaks **A** to **D** are related chemically, but only Peak **C** and **D** induces corn plants to release volatiles.

IMPORTED FIRE ANT

AND

HOUSEHOLD INSECTS

CRIS - 6615-32000-026-00D--Integrated Control of Insect Pests in an Urban Environment with Emphasison Roaches, Fleas, and Ants

CRIS - 6615-32000-028-00D--Fire Ant Ecology and Management

CRIS - 6615-32000-029-00D--Biological Control of Fire Ants

CRIS 6615-320000-026-00D

PILOT STUDY IN HOMES OF ASTHMATICS IN BALTIMORE ON SPATIALLY-BASED APPROACH TO ENVIRONMENTAL ASSESSMENT OF GERMAN COCKROACHES AND ATTENDANT ALLERGENS

R.J. Brenner[1], R. Tafts[2], D.A. Focks[1], G. Wiechmann[1], M.C. Anderson[2], E. Horowitz[3], D. Milne[1], J. Westmoreland[1], C. Hulme[2], T. Quander[2], and A. Togias[3]
[1] USDA-ARS, Gainesville, FL; [2] FDA, Bethesda, MD; [3] Johns Hopkins School of Medicine, Baltimore, MD

Objectives: Methods of assessing the presence of cockroaches and their attendant antigens in the environment are critical for the medical profession in evaluating asthma, and for the medical entomologist in developing and demonstrating reduced-risk management strategies. Preliminary studies in experimental structures at ARS in Gainesville resulted in the development of a polyclonal cockroach antigen detection system for assessing spatial distribution of cockroach antigens (allergens) for subsequent mitigation interventions. This field trial tested the use of this system in actual homes, the variability of assays, as a precision targeting strategy in homes of asthmatics to determine the impact of pest mitigation and professional cleaning on antigen load.

Methods: German cockroach distributions were determined using sticky traps baited and placed overnight. Spatial analysis was used to determine distributions for each floor of two homes selected because of a history of cockroach infestation. A rabbit-anti-cockroach polyclonal ELISA inhibition system was developed to detect the presence of cockroach-associated antigen. Reference swabs were taken from 100 cm^2 surface areas (petri dishes) where a known quantity of cockroaches resided for a specific amount of time. About 50 environmental samples were taken from each floor of each home. Assays were compared in two labs among five operators to develop statistical limits of the assay. Data were used in ArcView GIS to generate antigen load maps. A "blind" professional cleaning was conducted, and post-intervention environmental samples were taken and analyzed.

Results: Cockroaches were found only in one home, and infestations were heaviest in upstairs bedrooms (Fig. 1a). Foci were identified and a vacuum was used to remove cockroaches; residual bait was applied to foci. Antigen levels in the home without current infestation were below 150 cockroach-hr equivalents (c-h equiv); no recent asthmatic episodes were reported, and no intervention was conducted. In the infested home, antigen assays revealed values as high as 2200 c-h equiv; approximately 85% of the estimated antigen load was contained in less than 30% of the floor space (Fig 1b), corresponding to areas where human exposure was probable. Residents had reported asthmatic episodes within the past month. Post cleaning assays revealed areas where antigen load had decreased, and other areas (inadequately cleaned) where antigen load had increased (Fig. 1c). Levels in the carpet were reduced while those on non-floor hard surfaces were static or higher (Fig. 2), suggesting that improper rinsing of cleaning rags redistributed antigens. Variability in results among assay technicians and laboratories was negligible. We conclude that the antigen detection system allowed rigorous characterization of antigen loads, and that a standardized cleaning system could be developed and verified for efficacy.

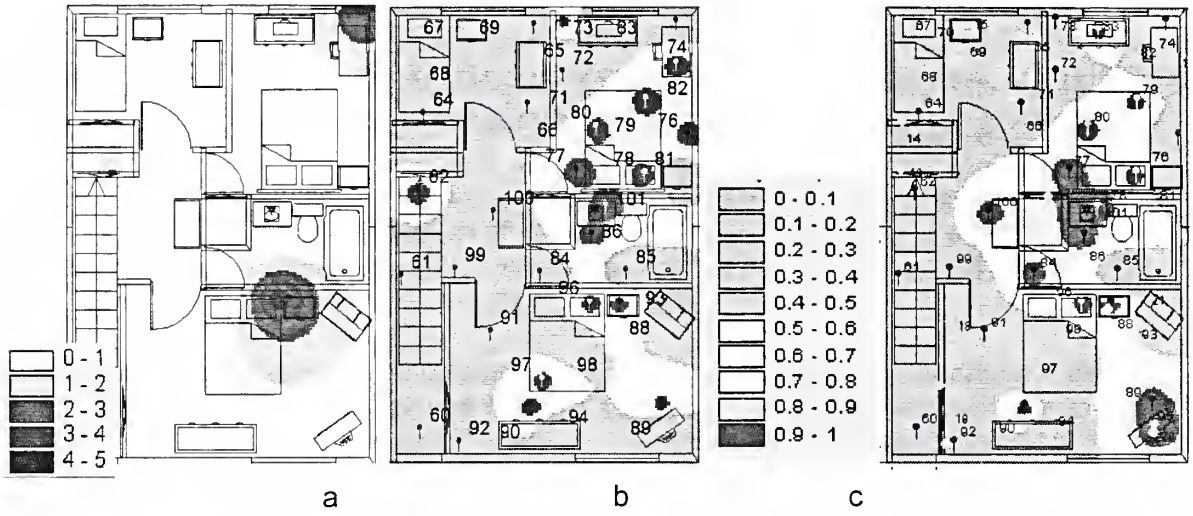

Figure 1. Cockroach distribution (no.) based on sticky traps (a), and probability contours showing distribution of cockroach antigen before (b) and after cleaning (c). Numbered symbols show locations of antigen samples. Precleaning map reveals foci accounting for 85% of antigen load (values > 54 cockroach-hour equivalents); postcleaning map shows distribution of foci still >54 c-h equiv. Notice that carpeted area at top of stairs improved (location 62), but improper cleaning procedures resulted in some increased levels elsewhere (e.g., locations 89, 83, 100).

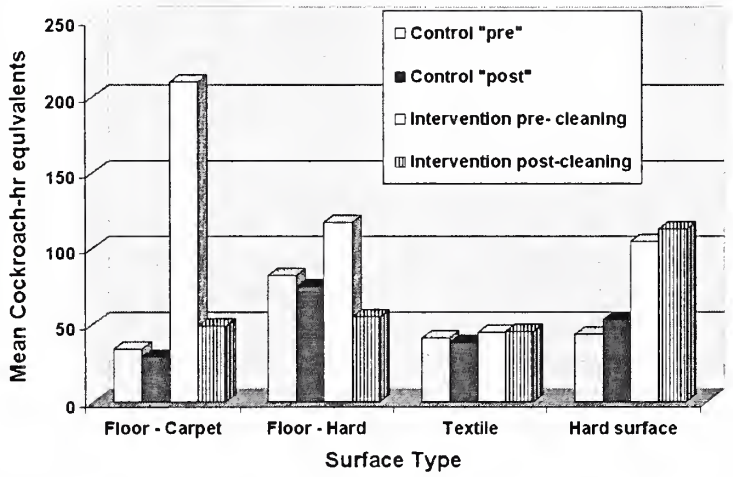

Figure 2. Comparison of mean antigen load (c-hr equiv) by surface type before and after cleaning (intervention). No cleaning was undertaken in control home; values reflect independent antigen samples taken 2 days apart. Only floor-carpet values for intervention are statistically significant ($p<0.05$).

CRIS 6615-32000-026-00D

TRANSMISSION THRESHOLDS FOR DENGUE IN TERMS OF *AEDES AEGYPTI* PUPAE PER PERSON AS A FUNCTION OF TEMPERATURE AND HERD IMMUNITY

D.A. Focks and R.J. Brenner[1]

Objectives: The expense and ineffectiveness of drift-based insecticide aerosols to control dengue epidemics has led to suppression strategies based on eliminating larval breeding sites. The present work estimates transmission thresholds for dengue based on an easily-derived statistic, the standing crop of *Aedes aegypti* pupae per person in the environment. These results also should be useful in anticipating the consequences of proposed climate change.

Methods: The notion of thresholds is based on two concepts: *the mass action principal*- the course of an epidemic is dependent on the rate of contact between susceptible hosts and infectious vectors, and *threshold theory*- the introduction of a few infectious individuals into a community of susceptibles will not give rise ɔ an outbreak unless the density of vectors exceeds a certain critical level. We used the validated transmission models CIMSiM/DENSiM developed at CMAVE to estimate thresholds as a function of levels of pre-existing antibody levels in human populations, ambient air temperatures, and size and frequency of viral introduction.

Results: Threshold levels ranged between about 0.5 and 1.5 *Ae. aegypti* pupae per person for ambient air temperatures of 28°C and initial seroprevalences ranging between 0 to 67% (for other temperatures, see Table 1). Surprisingly, the size of the viral introduction used in these studies, ranging between 1 and 12 infectious individuals per year, was not an especially significant determinant of the threshold (Figure 1).

The ratio of *Ae. aegypti* pupae to human density has been observed in limited field studies to range between 0.3 and >60 in 25 sites in dengue-endemic or dengue-susceptible areas in the Caribbean, Central America, and Southeast Asia. If, for purposes of illustration, we assume an initial seroprevalence of 33%, the degree of suppression required to eliminate the possibility of summertime transmission in Puerto Rico, Honduras, and Bangkok was estimated to range between 10 and 83%; however in Mexico and Trinidad, reductions of >90% would be required. A clearer picture of the actual magnitude of the reductions required to eliminate the threat of transmission is provided by the ratio of the observed standing crop of *Ae. aegypti* pupae per person and the threshold. For example, in a site in Mayaguez, Puerto Rico, the ratio of observed and threshold was 1.7- meaning roughly that about 7 out of every 17 breeding containers would have to be eliminated. For Reynosa, Mexico, with a ratio of ca. 10, 9 containers out of every 10 would have to be eliminated. For sites in Trinidad with ratios averaging ca. 25, the elimination of 24 of every 25 would be required. With the exceptions of Cuba and Singapore, no published reports of sustained source reduction efforts have achieved anything near these levels of reductions in breeding containers.

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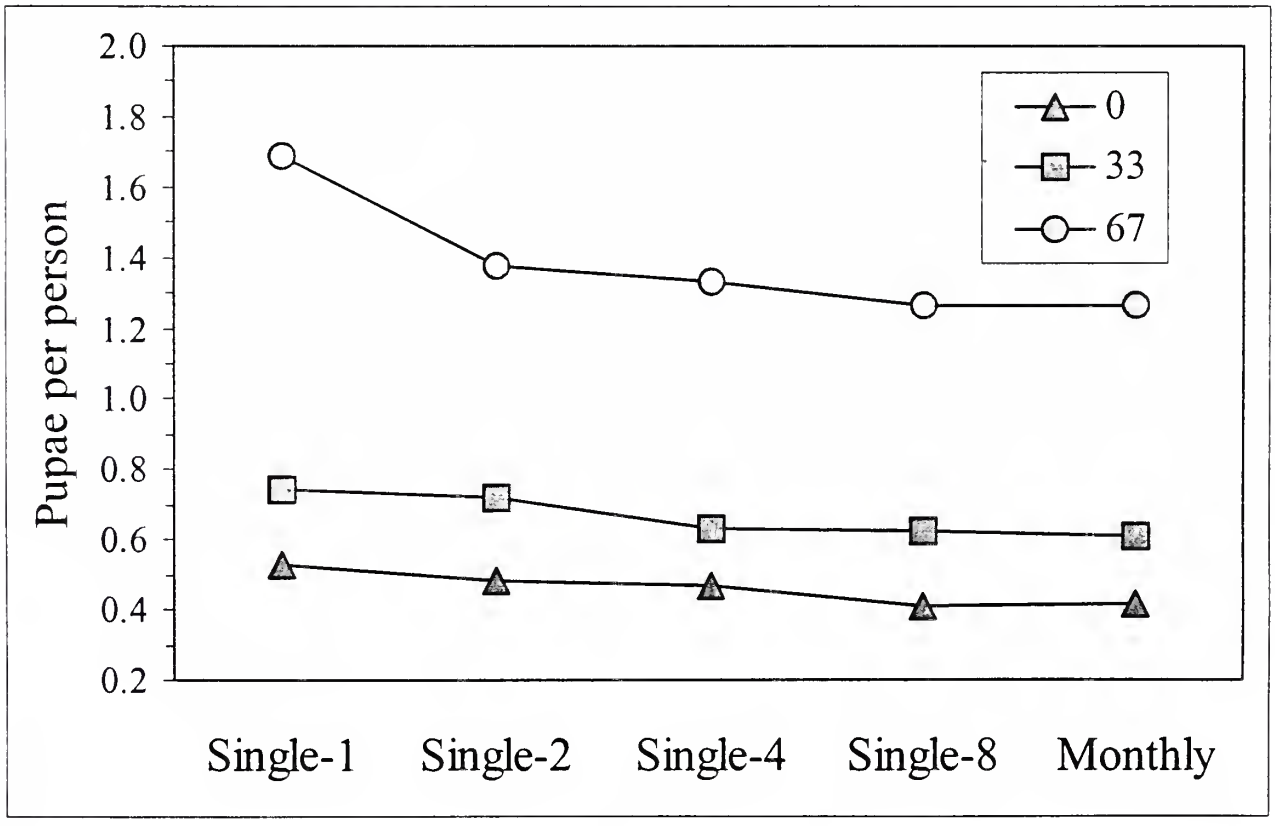
[1] Research reported in this paper was supported in part by funds from Pollution Prevention Project No. 1053, Strategic Environmental Research and Development program (SERDP) and EPA-USDA Interagency Agreement No. DW12937600-01-0.

Table 1. Estimated number of *Ae. aegypti* pupae per person required to result in a 10% or greater rise in seroprevalence of dengue antibody during the course of a year resulting from 12 monthly viral introductions of a single viremic individual (Monthly). Because the model is stochastic, in a series of simulations in DENSiM, these values resulted in a 10% or greater rise in prevalence ca. 50% of the time.

Temperature (°C)	Transmission threshold by initial seroprevalence of antibody		
	0%	33%	67%
22	7.13	10.70	23.32
24	2.20	3.47	7.11
26	1.05	1.55	3.41
28	0.42	0.61	1.27
30	0.10	0.15	0.30
32	0.06	0.09	0.16

Figure 1. Transmission thresholds at various temperatures for 12 monthly introductions (Monthly) (Table 1), or for a single introduction on day 90 of the year of 1, 2, 4, or 8 viremic individual(s) (Single-1, -2, -4, and –8) by initial seroprevalence of antibody (0, 33, 67%). Again, these are the estimated numbers of *Ae. aegypti* pupae per person required to result in a 10% or greater rise in seroprevalence of dengue antibody during the course of a year. In a series of simulations in DENSiM, these values resulted in a 10% or greater rise in prevalence ca. 50% of the time.



CRIS 6615-320000-026-00D

TOXICOLOGICAL AND BIOCHEMICAL STUDIES WITH FIELD POPULATIONS OF THE GERMAN COCKROACH

S.M. Valles

Objective: To determine the feasibility of using detoxification enzyme activity toward surrogate substrates to identify insecticide resistance magnitude in German cockroaches.

Methods: Adult males of each strain were anesthetized with CO_2 (12) and treated topically with insecticide in 1 µl of acetone applied to the first abdominal sternite in five concentrations causing >0% and <100% mortality. Synergists PBO (100 µg per cockroach) or DEF (30 µg per cockroach) were applied to the first abdominal sternite 1 hr before insecticide application. Mortality was recorded at 24 hr. Microsomal epoxidase activity was measured by the epoxidation of aldrin to dieldrin. Microsomal *O*-dealkylase activity was measured using methoxyresorufin as substrate. Total cytochrome P450 was determined by the method of Omura and Sato. The molar extinction coefficient of 91 mM^{-1} cm^{-1} was used to calculate the specific content of cytochrome P450. Glutathione *S*-transferase activity was measured with 1-chloro-2,4-dinitrobenzene (CDNB) as substrate. Enzyme activity was expressed as nmol CDNB conjugated per min per mg protein using the molar extinction coefficient of 9.6 mM^{-1} cm^{-1} for the product *S*-(2,4-dinitrophenyl)glutathione. General esterase activity was measured in individual adult male cockroaches using a microtiter plate assay. Individual cockroaches were placed into a 1.5 ml microfuge tube with 1 ml of 0.1 *M* sodium phosphate buffer, pH 7.0, and homogenized with a motor-driven Teflon pestle. The homogenate was centrifuged at 0-4 °C for 10 minutes at 10,000 *g*. The supernatant was then diluted 100-fold in sodium phosphate buffer, pH 7. The incubation mixture contained 100 µl of the diluted supernatant and 0.5 m*M* *p*-nitrophenyl acetate (PNPA) in a final volume of 200 µl. Assays were monitored at 405 nm using a SpectraMax 250 microtiter plate spectrophotometer. Formation of the product, *p*-nitrophenol, was measured for 15 minutes at 15 second intervals.

Results: Resistance levels ranged from 3- to 159-fold for cypermethrin, 2- to 88-fold for permethrin, 4- to 55-fold for λ-cyhalothrin, 5- to 33-fold for propoxur, and 3- to 19-fold for chlorpyrifos. Synergists affected cypermethrin resistance to varying degrees depending on the strain. PBO pretreatment decreased resistance in only 5 strains, but caused an increase in 7 strains. Conversely, DEF pretreatment reduced the resistance level in 10 of the strains and increased the resistance level in only 2 strains. All field strains exhibited significantly higher microsomal oxidase, glutathione *S*-transferase and esterase activities toward surrogate substrates as compared with the insecticide susceptible strain. However, levels of cytochrome P450 content, aldrin epoxidase activity, methoxyresorufin *O*-demethylase activity, and glutathione *S*-transferase activity were not correlated with pyrethroid resistance suggesting that these activities are poor indicators of pyrethroid resistance magnitude. Interestingly, significant correlations were found between general esterase activity and cypermethrin (P = 0.002), permethrin (P = 0.007), cyhalothrin (P = 0.002), and propoxur (P = 0.001) resistance levels. The data support the conclusion of esterase involvement in cypermethrin resistance determined by synergist (DEF) bioassay.

CRIS 6615-320000-026-00D

VARIATION IN HYDRAMETHYLNON SUSCEPTIBILITY AMONG INSECTICIDE-RESISTANT GERMAN COCKROACHES

S.M. Valles and R.J. Brenner

Objective: Measure hydramethylnon susceptibility among multi-resistant strains of German cockroach.

Methods: Hydramethylnon dose-mortality relationships were conducted on 8 German cockroach strains (Orlando, Sacramento, Union 507, Union 511, Pinellas 214, Malo, Marietta, and Levy 616). Adult male cockroaches were removed from their rearing tubs and placed into separate 4 L glass containers with cardboard harborage and one cotton-stoppered plastic vial (50 ml) of tap water. After a period of starvation, individual cockroaches were placed into 4 ounce Mason jars with the interior lip coated with a 2:3 mixture of mineral oil and petroleum jelly to prevent escape. A quartered plastic weigh boat (1.2 x 1.2 cm) containing a 1.086 mg portion of Siege® gel bait and a cotton-stoppered microfuge tube of water were subsequently placed into each jar. The cockroaches were placed immediately into an incubator at 26° C. Twelve hours after the cockroaches were placed onto the bait, they were scored for consumption. Cockroaches that did not consume the bait portion completely were excluded from the toxicity bioassay. Approximately 100 mg of rodent diet (PMI Feeds, St. Louis, MO) was placed into the remaining jars. At least 5 doses of hydramethylnon (in Siege® gel bait) causing >0% and <100% mortality were used against each strain. Three replications containing 10 cockroaches per dose were conducted. Controls were fed Siege® gel bait devoid of hydramethylnon. Mortality, which was scored as the inability of a cockroach to right itself within 15 seconds after being flipped onto its dorsum, was assessed 96 h after placement on the gel bait.

Results: Results of the hydramethylnon toxicity and consumption bioassay against the Orlando and 7 insecticide resistant field strains are summarized in Table 1. Despite the fact that all of these field strains were resistant to pyrethroid, organophosphate, and carbamate insecticides, they were more susceptible to hydramethylnon than the insecticide-susceptible Orlando strain. However, the Union 511 and Malo strains consumed significantly fewer bait portions as compared with the Orlando strain. Despite lower hydramethylnon tolerance as compared with the Orlando strain, significant variation (2.4-fold) in hydramethylnon susceptibility was observed among the field strains. Although the bioassay data did not indicate physiological resistance among these strains, behavioral resistance may be present in some strains. Significantly fewer individuals of the Union 511 and Malo strains consumed the 1.086 mg Siege® gel bait portions. This may indicate that these strains are developing an aversion to a component in the Siege® gel bait.

Table 1. Siege gel bait consumption and hydramethylnon toxicity among multi-insecticide-resistant German cockroach strains

Strain	n^a	% Consumed[b]	LD_{50}(95% CI)[c]	Slope ± SE	χ^2	TR[d]
Orlando	150	98.0 ± 0.0013	1.53 (1.40-1.66)	6.51 ± 1.13	0.11	1
Sacramento	180	98.9 ± 0.0078	0.45 (0.41-0.49)	5.41 ± 0.76	1.31	0.29
Union 507	150	96.7 ± 0.015	0.64 (0.53-1.00)	2.97 ± 0.76	0.59	0.42
Union 511	150	83.3 ± 0.031*	0.68 (0.61-0.76)	5.67 ± 1.00	0.73	0.45
Pinellas 214	150	94.0 ± 0.019	0.77 (0.67-0.93)	4.17 ± 0.78	3.26	0.50
Malo	150	93.3 ± 0.020*	0.81 (0.74-0.94)	5.10 ± 0.98	5.66	0.53
Marietta	150	96.0 ± 0.016	0.94 (0.84-1.17)	4.65 ± 1.05	1.13	0.62
Levy 616	150	98.0 ± 0.0013	1.10 (0.92-1.29)	3.43 ± 0.96	0.14	0.72

[a] Total number of individual cockroaches provided a 1.086 mg portion of Siege bait.

[b] Percentage of the total number of cockroaches used in the bioassay that consumed the 1.086 mg portion of Siege gel bait. An asterisk denotes significant difference ($P < 0.05$) by Chi-Square analysis as compared with the Orlando strain.

[c] µg hydramethylnon/cockroach.

[d] Tolerance ratio, LD_{50} field strain/LD_{50} Orlando strain.

CRIS 6615-320000-026-00D

CYTOCHROME P450 MONOOXYGENASE ACTIVITY IN THE DARK SOUTHERN SUBTERRANEAN TERMITE.

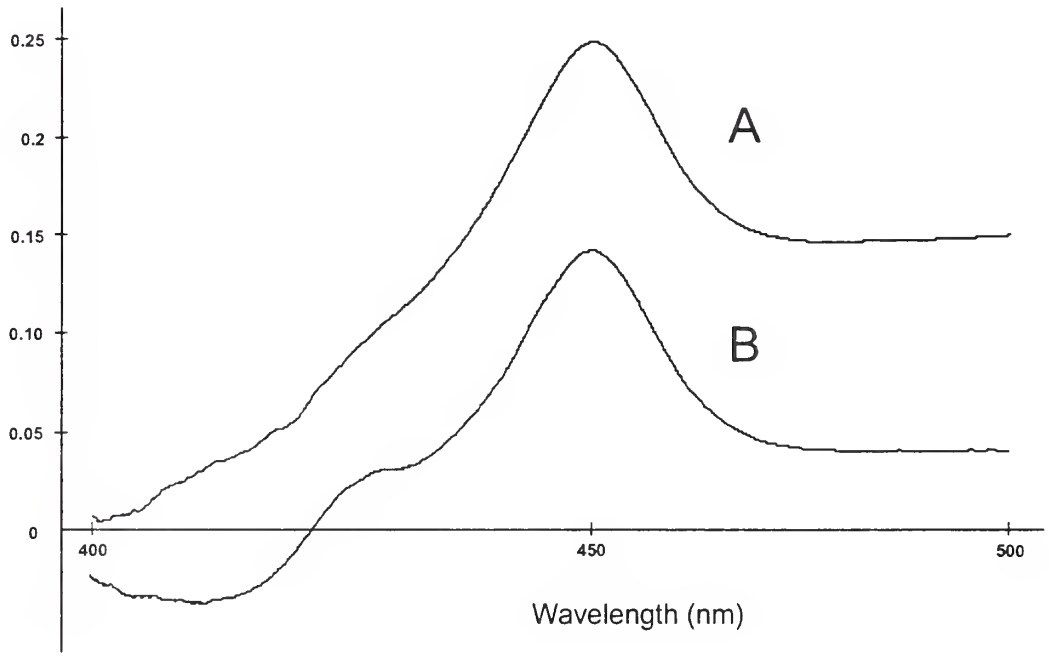
S.M. Valles, W.L.A. Osbrink, F.M. Oi, R.J. Brenner, and J.E. Powell.

Objective: Characterize cytochrome P450 enzyme activity in *Reticulitermes virginicus*.

Methods: Four colonies of R. virginicus were collected from field sites in Florida. Colony 02 was collected 25 September, 1997 from a wooden porch at Jacksonville Naval Air Station, Jacksonville, FL. Colonies 21 and 26 were collected 20 August, 1997 from pine logs within a stand of pine trees at the Alachua County fair grounds, Gainesville, FL. Colony 71 was collected 29 October, 1997 from pine logs within a stand of pine trees at the University of Florida's natural area teaching laboratory, Gainesville, FL. Microsomes were prepared from a mixture of soldier and worker termites (1 g) in 40 ml of a protective buffer (0.1 M sodium phosphate buffer, pH 7.5, containing 10% glycerol, 0.1 mM dithiothreitol, 1 mM ethylenediaminetetraacetic acid, 1 mM phenylmethylsulfonyl fluoride, and 1 mM phenylthiourea)by using a motor-driven Teflon pestle and glass mortar. Microsomal epoxidase activity was measured by the epoxidation of aldrin to dieldrin. When conducting the temperature experiments, incubations occurred at 18, 22, 26, 30, and 34°C. When time course experiments were conducted, all components were incubated in a common vessel (250-ml flask) at 22°C for 6, 9, 12, 15, and 18 min with 0.25 mg of microsomal protein per incubate. Aliquots (5 ml) were removed by pipette at the appropriate times. Microsomal O-dealkylase activity was measured using the substrates methoxyresorufin, ethoxyresorufin, and pentoxyresorufin. Total cytochrome P450 and cytochrome b_5 were determined by the method of Omura and Sato. Cytochrome *c* reductase activity was determined by the reduction of cytochrome c.

Results: Aldrin epoxidase activity required NADPH and was inhibited by carbon monoxide and piperonyl butoxide ($I_{50} = 4.72 \pm 0.31 \times 10^{-6}$ M) indicating that the enzyme(s) involved was a cytochrome P450 monooxygenase. Aldrin epoxidase activity was highest at 22°C and pH 7.2. Also, the activity was linear up to 0.5 mg of protein per incubate and increased with reaction time up to 15 min. Although neither substrate nor cofactor was found to be a limiting factor, aldrin epoxidase activity failed to produce a linear response with respect to time at temperatures above 22° C indicating enzyme inhibition. Although increased incubation temperature above 22° C resulted in decreased aldrin epoxidase activity, similar heat treatments did not result in a concomitant increase in cytochrome P420. Significant variation (2.7-fold) in aldrin epoxidase activity was observed among 4 R. virginicus colonies collected from different locations in Florida. Additionally, cytochrome P450 and b_5 content, cytochrome c reductase, and methoxyresorufin O-demethylase activities were measured in R. virginicus.

Figure 1. Reduced carbon monoxide spectrum of microsomes prepared from *Reticulitermes virginicus* soldiers and workers (colony 21). (A) Measured immediately and (B) after a 10 min incubation at 34°C.



CRIS 6615-32000-028-00D

ADOPTION OF NEWLY MATED QUEENS BY QUEENLESS POLYGYNE AND MONOGYNE *SOLENOPSIS INVICTA* COLONIES

R.K. Vander Meer[1] and L.E. Alonso[1,2]

Objective: Large numbers of imported fire ants (IFA) currently infest millions of hectares in the southern states. The IFA is a medical, agricultural and environmental pest species. A multiple queen form (polygyne) of the IFA proliferates in infested areas and is difficult to control. The mechanism of polygyne colony formation and maintenance of existing polygyne populations is of considerable interest. Our objective is to determine how the decrease in conspecific aggression observed when workers become queenless affects newly mated queen acceptance and survival.

Methods: The monogyne or polygyne status of collected test colonies was determined by standard methods. Monogyne and polygyne queenless worker groups were maintained in the laboratory by the methods reported for the maintenan`e of normal colonies. Newly mated queens (NMQs) were collected immediately after mating flights from around the Gainesville, FL area. NMQs were introduced into queenless colony fragments within three hours of their mating flight. Three sets of monogyne and polygyne workerless units (N=10 each) were used for NMQ introductions. Two sets had NMQs introduced at three different times and the third had NMQs introduced only once. Colony test units were inspected for queen survival each day for 73 days. Controls consisted of individual NMQs and NMQs in groups of five, founding claustrally, without workers. At the end of the experiment weights were measured for workers and brood from each colony fragment group and control group, and for each living NMQ from the colony fragments and control groups.

Results: Every queenless worker group from monogyne and polygyne colonies readily adopted all NMQs introduced 16 days after the worker groups were collected from the field and made queenless. Once NMQs were adopted, worker aggressiveness toward subsequently introduced NMQs returned to high levels in both monogyne and polygyne worker groups, demonstrating the influence of their new queen(s). Within 48 hours of NMQ introduction, 91 and 89% of 200 NMQs were executed when added to 20 monogyne and 20 polygyne worker groups, respectively, that had previously adopted at least one queen. In contrast, 48 hours after initial NMQ introductions into queenless worker units only 10 and 7% queen mortality was observed for monogyne and polygyne worker units, respectively. Most of the monogyne worker groups (28 of 30, 93.3%) adopted and maintained at least one NMQ by the end of the experiment. All 30 polygyne queenless worker units accepted and maintained at least one NMQ. Colonies founded by groups of five NMQs produce more workers than their single NMQ counterpart. However, we found that NMQs adopted into queenless worker groups have a 10 to 20 fold advantage in biomass production over NMQs that found colonies individually or in groups. We conclude that queenless workers can perpetuate their colony by NMQ adoption and a small percentage will accept and maintain multiple NMQs.

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CRIS 6615-32000-028-00D

POWERFUL QUEEN INFLUENCE ON CONSPECIFIC FIRE ANT, *SOLENOPSIS INVICTA*, AGGRESSION

R.K. Vander Meer[1] and L.E. Alonso1,2

Objective: The imported fire ant (IFA), *Solenopsis invicta,* currently infests over 150 million hectares in Puerto Rico and twelve southern states from Texas to Virginia. The IFA is a medical, agricultural and environmental pest species. A multiple queen form (polygyne) of the IFA has been proliferating in infested areas over the last two decades. A major behavioral difference between monogyne and polygyne populations is their aggressive behavior toward conspecifics. Monogyne fire ant workers from fully functional colonies (queenright) are highly aggressive toward con- and heterospecific intruder worker ants, whereas polygyne colony workers are aggressive toward heterospecific intruders but not conspecifics. Thus, each monogyne colony in a population is an enclave, defending its territory, while colonies in a polygyne population exhibit exchange of workers and food and probably queens and brood. The exception to this behavioral dichotomy is that the two fire ant forms are highly aggressive toward heterospecific workers and to conspecific NMQs. The objective of this study is to determine the influence of the queen on nestmate recognition by observing and measuring worker aggression with and without queens for both monogyne and polygyne colonies.

Methods: All fire ant colonies, colony fragments, and newly mated queens (NMQs) were obtained from the Gainesville, FL area. The monogyne or polygyne status of collected test colonies was determined by standard methods. Monogyne and polygyne queenless worker groups were maintained in the laboratory by the methods reported for the maintenance of normal colonies. The aggression bioassay is well documented in the literature.

Results: We found that queenright monogyne colonies were highly aggressive toward conspecific workers from polygyne and other monogyne colonies, as well as toward NMQs. However, queenright polygyne workers were not aggressive toward workers from either colony form, but were highly aggressive toward newly mated queens. In sharp contrast, workers from monogyne colonies that had their queen removed became non-aggressive toward all types of conspecific workers, as well as NMQs. Queenless polygyne workers maintained their non-aggressive behavior toward other workers, but now were also non-aggressive toward NMQs. Aggressive interactions were measured as a function of how long workers were queenless. The results showed a rapid decrease in aggressivity, such that within one week of becoming queenless worker aggression decreased from lethal to sublethal levels. Heterocolonial monogyne worker aggression continued to decrease until becoming indistinguishable from those of non-aggressive polygyne workers. All queenless workers groups that were non-aggressive toward conspecific workers were highly aggressive toward heterospecific workers (*S. geminata*).

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Effect of red imported fire ants on Florida Grasshopper Sparrows.

D.P. Wojcik [1] , C.R. Allen [2] , and E.A. Forys [3]

Objectives: 1. To determine the distribution and abundance of red imported fire ant (RIFA) throughout the range of the Florida Grasshopper Sparrow (FGS) at the Avon Park Air Force Range, FL. 2. To determine the relationship between RIFA abundance and the abundance of FGS prey. 3. To determine the effects of different burn regimes on RIFA density. 4. To determine the seasonal variation in RIFA distribution and abundance.

Methods: 12 plots each 20-100 hectares in size that had been established for other FGS studies were used in this study. RIFA densities were determined at each site by placing ARS standardized, multiple-species ant baits at 8 permanent sampling locations in each plot (2 baits/ location), for 192 samples. Baits were collected after one hour and the number of ants recruited to bait in that period of time quantified. Additionally, at all sites, 2 pitfalls were placed at each permanent sampling location, separated by 2 meter, for a total of 192 pitfall traps. Pitfall traps were collected after 7 days. Light trap samples were taken to collect biomass samples in each of the 12 plots. One light trap was placed in each plot at ground level and operated from sunset to sunrise. Sweep net samples were taken in October 1998 to provide additional biomass samples. The biomass samples will be taken and processed by APHIS personnel.

Results: Samples were taken in June 1997, October 1997, May 1998 and October 1998. RIFA were dominant in Delta trail plots while OQ plots with exceptions tended not to have RIFA, with exceptions. RIFA abundance was strongly negatively correlated with both native ant abundance and native ant richness. The next sampling period is scheduled for Spring 1999.

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CRIS 6615-32000-028-04R

The effect of red imported fire ants (*Solenopsis invicta*) on *Gopherus polyphemus* at Camp Shelby, Mississippi.

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Objectives: 1. To determine the Mississippi National Guard abundance of RIFA on selected sites containing gopher tortoise colonies at Camp Shelby Training Site. 2. To determine the relationship between red imported fire ant abundance and gopher tortoise hatchling survivorship. 3. To determine the relative abundance of red imported fire ants in relation to different habitat disturbance regimes (including baiting). 4. To determine the level of red imported fire ant utilization of gopher tortoise burrow systems, and impacts on invertebrate burrow commensals.

Methods: For a 4-year project, 6 pairs of study sites, each 20-40 hectares in size, containing resident tortoise colonies were established; 6 sites were treated with Logic® at label rates and 6 sites were used as untreated checks. *S. invicta* densities were determined at each site by placing ARS standardized, multiple-species ant baits along 2 transects (20 baits/ transect) through each site. Baits were collected after one hour and the number of ants recruited to baits in that period of time quantified. Additionally, in selected sites, *S. invicta* mounds were counted using a .1 ha radius circular plot, and mound densities determined. While quantifying *S. invicta* levels, sampling to determine *S. invicta* use of burrow systems and the impact of *S. invicta* on burrow commensals, as accomplished using baits placed in burrows and a suction apparatus. Gopher tortoise reproduction was monitored by Camp Shelby Training Site personnel.

Results: Pretreatment samples were taken in July-August 1997 and April-May 1998. RIFA were present in all plots and the ant populations were used to determine plot pairing. Plots were treated with Logic® at label rates at the end of May 1998. The first post-treatment samples were taken in August-September 1998. Field observations during bait sampling and selected circle counts indicated excellent control with substantial reduction in RIFA populations. The next post-treatment sampling is scheduled for Spring 1999.

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FIELD STUDIES OF THE PARASITIC ANT, *SOLENOPSIS DAGUERREI* ON FIRE ANTS IN ARGENTINA

L.A. Calcaterra, J.A. Briano and D.F. Williams

Objectives: The workerless, parasitic ant, *Solenopsis daguerrei* has been considered as a potential candidate for the biological control of the imported fire ant, *Solenopsis invicta* in the U.S. The ant occurs only in South America and with the cooperation of ARS's South American Biological Control Laboratory in Buenos Aires, Argentina, our objective is to conduct field research on this insect for it's potential use in the U.S. against *S. invicta*. The specific objective of this project is to study the detrimental effects of *S. daguerrei* on native Argentina fire ants, *S. richteri,* and also aspects of the parasitic ant's biology.

Methods: The study was conducted in pastures on 5 ranches at San Eladio, Buenos Aires Province that had an *S. daguerrei* abundance of 7 percent abundance (very high) in the local fire ant *(S. richteri)* population. A total of 2580 fire ant colonies were opened with a shovel and examined for the presence of *S. daguerrei.* A comparison was made of fire ant mound densities, mound volumes, colony compositions, incidence of polygyny, number of queens per colony, queen weight and the presence and abundance of brood and sexuals in the parasite area versus a parasite-free area. In addition, the presence of sexuals, abundance and weight of queens, and sex ratios were determined for *S. daguerrei.*

Results: *S. daguerrei* occurred in 1.2 to 23 % of the fire ant colonies at 21 collecting sites. The mound density at parasitized sites was significantly lower than in parasite-free sites (161 versus 239 mounds per hectare. Parasitized colonies had significantly fewer host queens than nonparasitized ones (2.9 versus 5.5 queens per colony). The percentage of colonies with worker brood was significantly lower in the fall in parasitized colonies than in nonparasitized ones. Also, worker brood was significantly less abundant in the fall and spring in parasitized colonies. A short delay in the production of sexual brood by the host was observed in parasitized colonies. These detrimental effects look very promising but the studies should be evaluated over a longer period and in other areas of Argentina. A much better understanding of the host and parasite is required before we will be able to use *S. daguerrei* as a biological control agent for imported fire ants in the U.S.

CRIS 6615-32000-029-00D

FIELD RELEASES OF AN ENTOMOPATHOGEN OF IMPORTED FIRE ANTS IN THE UNITED STATES

D.H. Oi and D.F. Williams

Objectives: To infect imported fire ant colonies in diverse geographic locations with the entomopathogen, *Thelohania solenopsae,* and to determine its impact on fire ant populations. This project directly supports the National Fire Ant Strategy, a new multi-state effort lead by ARS under the auspices of the Southern Legislative Conference. Goals of this strategy are to develop, test, and implement biologically-based technologies for managing fire ants, and to subsequently develop customized regional integrated management strategies. Originally identified in Brazil in 1973, *T. solenopsae* is the most common pathogen of fire ants in South America. It was discovered in the U.S. in 1996 from fire ant colonies in Florida, Mississippi and Texas.

Methods: Fire ant brood infected with a microsporidian entomopathogen, *T. solenopsae*, was introduced into imported fire ant colonies at paired inoculation and control plots within 10 states (AK, OK, MS, LA, TN, SC, AL, GA, NC, and FL). Baseline data on natural infections of *T. solenopsae* were obtained from worker ant samples. Fire ant populations were assessed by estimating colony sizes using the USDA population index method. The presence of fire ant and non-fire ant species within each plot was determined by counting and identifying foraging ants attracted to a multiple ant species attractant (patent pending) and ants collected in pitfall traps. Post-inoculation evaluations are currently being made at 2 month intervals. Cooperators from state universities, state departments of agriculture, and USDA-APHIS are collecting data for the post-inoculation evaluations.

Results: Study sites were negative for *T. solenopsae* before inoculations were made. While non-fire ant species were collected from most plots, fire ants were the predominant ant species. Two months after inoculations, an infection was detected from one sample in Mississippi, while other sites were negative, and fire ant populations remained unchanged. The low infection rate was expected based on preliminary studies conducted in Florida. Evaluations at four months are currently underway. Samples from Arkansas have resulted in infections being detected in three of the inoculated mounds and in three uninoculated mounds, thus indicating the pathogen may be spreading naturally. It is still too early to determine the impact of this pathogen on field populations of fire ants. Laboratory studies have indicated that this disease slowly debilitates fire ant queens with significant declines in colony populations being observed after six months.

CRIS 6615-32000-29-11D

RELEASE OF THE DECAPITATING FLY *PSEUDACTEON TRICUSPIS* (DIPTERA: PHORIDAE) FOR FIRE ANT BIOCONTROL IN THE SOUTHEASTERN UNITED STATES

S.D. Porter

Objectives: The objectives of this project are to develop techniques for mass rearing, successfully releasing. and assessing the effectiveness of the phorid fly *Pseudacteon tricuspis* as a biocontrol agent for imported fire ants in the southeastern United States.

Methods: We are releasing flies into test sites in several states. During FY 1997-1998, we released flies near Gainesville, FL, at 3 test sites (Hog Town Creek, Sept.-Oct. 1997; Morrill Farm, May-June and Aug.-Sept 1998; Airport, Sept.-Oct 1998). Decapitating flies were also released in Monticello, AR (Aug.-Sept 1998), Talladega, AL (June and Aug.-Sept.), and Durant, OK (Oct. 1998). Releases are being conducted at paired treatment and control sites so we will eventually be able to assess impacts on imported fire ant populations, once the fly populations have had time to build up and the native ant populations have had time to recover.

Results: Flies at the Hogtown Creek site in Florida appear to be permanently established in the field. They have been collected at this site every month for the past 12 months. During this time they have survived the winter and a severe summer drought. So far they do not appear to be expanding out of this site and it is too early to assess impacts on fire ant populations, although monitoring is progressing. A few first generation flies have been recovered at the Morrill Farm, site, but we will not know if they are permanently established until next spring. We hope to begin recovering field-reared flies from the airport site in November 1998.

Several field-reared flies have been recently observed at the Arkansas site (Oct. 1998). We are still awaiting results from the Alabama and Oklahoma sites.

We are working with the ARS lab in Starkville, MS to develop ways of further expanding our rearing capabilities so we can release at more sites in the coming year. Plans are being made for additional releases in Florida, Texas, Alabama, South Carolina, and Oklahoma. Additional sites may also be selected as resources permit.

MOSQUITO

AND

FLY

CRIS - 6615-32000-031-00D--Repellent Systems and Control Strategies for Mosquito/Vectors of Medical and Veterinary Importance

CRIS - 6615-32000-032-00D--Biological Control and Integrated Management of Bloodsucking and Nuisance Flies of Med/Ag/Vet Importance

CRIS 6615-32000-031-00D

REPELLENCY OF ESSENTIAL OILS TO MOSQUITOES

D.R. Barnard

Objective: On May 6, 1996 the USEPA (40 CFR 152.25 (g)) ruled on an exemption from regulation under FIFRA for certain minimum risk pesticides. The exempted products comprised 31 active ingredients ranging from castor oil to zinc metal strips and included 11 essential oils of plant origin. Currently, several commercially-available, 'natural' mosquito repellents intended for skin application contain essential oils from the EPA list in concentrations between 0.1 to 10%. The basis for using these concentrations has not been established, however, and there are no documented reports of mosquito repellency for mixtures of two or more essential oils. In this study I characterized the relationship between different concentrations of essential oils, and mixtures of oils, on human skin and the length of time mosquitoes were repelled.

Methods: White cedar oil, clove oil, peppermint oil, red thyme oil, and Bourbon geranium oil were selected for testing. Repellency for each oil was determined at the 5, 10, 25, 50, and 75% concentrations (in ethyl alcohol) and for the pure (100%) oil. Each test comprised the comparison of mosquito repellency for one concentration of an essential oil on a human volunteer with the repellency of 25% deet (*N,N*-diethyl-3-methylbenzamide) on the same volunteer. Tests were made against *Aedes aegypti* and *Anopheles albimanus*.

Results: Cedarwood oil was not repellent to mosquitoes and peppermint oil repelled *Ae. aegypti* only slightly. Thyme and clove oils provided 1½ to 3½ hr protection, depending on mosquito species and oil concentration. Clove oil repellency increased commensurate with oil concentration but none of the oils prevented mosquito bites when used at the 5% or 10% concentration. Against *Ae. aegypti*, variations in mean protection time among oil concentrations were significant for Bourbon geranium oil, clove oil, and thyme oil. Pure thyme oil was significantly more repellent to *Ae. aegypti* than were the lower concentrations . Both clove and thyme oils were repellent to *An. albimanus* and both oils provided the greatest protection at the 100% concentration. The repellency of clove oil to *An. albimanus* is remarkable given the inability to develop an effective synthetic repellent for this species over the years. Twenty-five percent clove oil provided a mean protection time of 75 min against *An. albimanus*; pure clove oil provided 213 min protection. These protection times are 2½ and 7 times as long, respectively, as that for 25% deet. The basis for this repellency is problematic. Eugenol, eugenol-acetate, and β-caryophyllene are the major constituents of clove oil but neither eugenol-acetate nor β-caryophyllene is repellent to *Ae. aegypti*, and neither has been tested for repellency to malaria mosquitoes. Except for the 75% clove + 25% thyme oil mixture, none of the oil combinations tested repelled *Ae. aegypti* as well as 25% deet. None repelled *Ae. aegypti* longer than the constituent oils alone. In contrast, repellency of each of the 4 oil mixtures to *An. albimanus* exceeded that for deet and was greater than the sum of the mean protection times for each constituent oil in every case except the 25% clove + 75% geranium oil mixture.

CRIS 6615-32000-031-00D

CHEMICAL BLENDS WITHOUT CARBON DIOXIDE PRODUCE HIGH LEVEL ATTRACTION OF *Aedes aegypti*

U.R. Bernier, D.L. Kline, D.R. Barnard, and K.H. Posey

Objective: Identify volatile chemicals, primarily those emanated from human skin, that activate and attract the Yellow Fever mosquito (*Aedes aegypti*). Formulate environmentally-safe blends of chemicals that attract this species with high efficiency. Use these attractant blends in combination with surveillance traps for more accurate information regarding the local mosquito populations that pose a threat to human and animal health. Possible future use in alternative means of mosquito control via the use of removal-trapping strategies.

Methods: A triple cage, dual port olfactometer was used to assess the attraction of 6-8 day old laboratory reared nulliparous female *Aedes aegypti*. Bioassays were conducted three times a day, at 08:30, 11:00, and 13:00 hours. Test cages were loaded, from fresh populations, with approximately 75 females. The mosquitoes were allowed to acclimate for approximately one hour prior to testing. Each test was conducted for a period of 3 minutes; the number of mosquitoes trapped in the baited and unbaited ports, and those remaining in the cage were counted. The data are then presented as a percentage of total mosquitoes in the cage that were attracted to the baited port. Various sized containers and vials were used to produce different emission rates of chemicals. The second port contained the same apparatus as the baited port, except that the chemical or blends were omitted.

Results: Many of the 125 chemicals screened for activity resulted in attraction at high chemical emission rates. Blends of multiple compounds provide collection efficiencies, measured by percent attraction, equal to or greater than that of the human hand. During study of *Aedes aegypti*, it appears that certain chemicals behave similarly, and that combinations of two types of these chemicals has a synergistic effect on the attraction of mosquitoes. For example, L-lactic acid is a known attractant and is the "base attractant" shown in **Figure 1**. Other compounds produce a similar attraction response to lactic acid in that they do not seem to highly excite mosquitoes. An example of a well known "activator" is carbon dioxide, but this compound is not used, nor necessary to be used for attraction of this mosquito. The activators shown in this figure created a high level of excitation in mosquitoes, with all or nearly all of them taking flight. The attraction was not as great as expected, considering the amount of activity observed in the cage. However, certain combinations of base attractant and activator resulted in a high level of excitation and well-oriented flight of the mosquitoes to the baited port. The collection efficiency for these mosquitoes is equivalent to, or greater than, the collection efficiency using the human hand. Attractant blends are being tested in the field to determine their effectiveness with wild mosquitoes. These reported olfactometer tests were conducted non-competitively, where a control or blank was used in the opposite port. A blend that is more attractive than the human hand does not exist; however, studies are currently underway to improve the effectiveness of these blends such that they approach the level of attraction produced by human emanations.

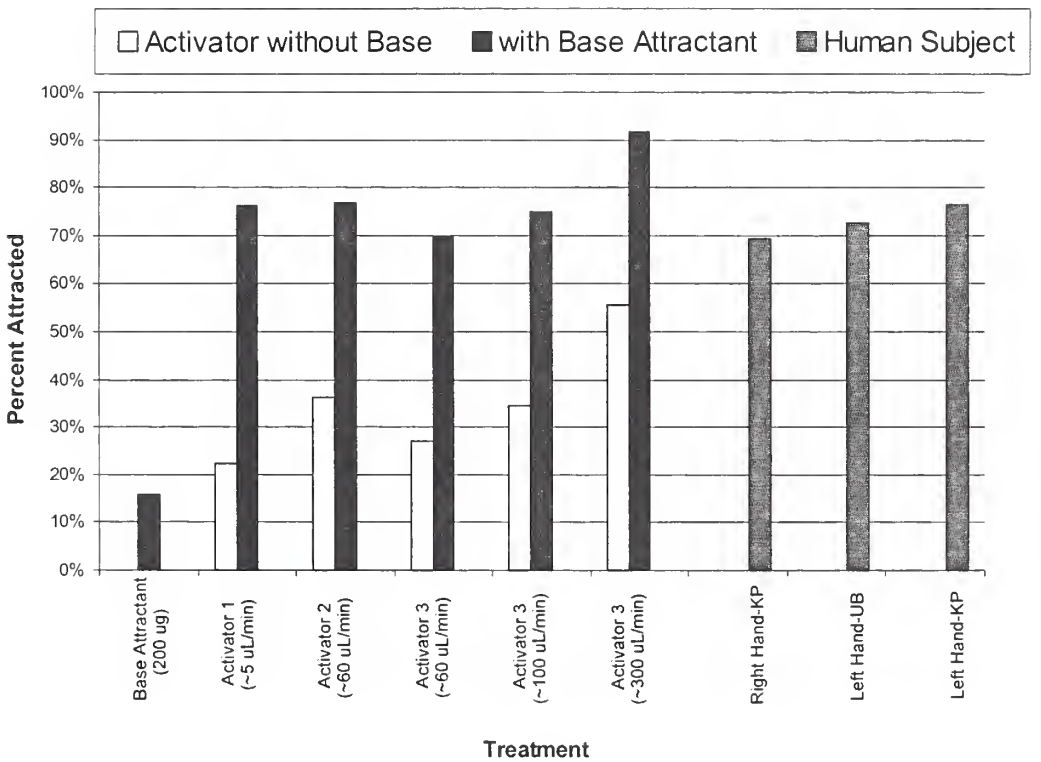

Figure 1. Percentage of *Aedes aegypti* attracted to L-lactic acid (base attractant), single compound activators, the synergistic combination of base attractant plus activator, and the human hand for two different subjects. The standard error of the mean for all chemical treatments is less than 2.2%. The standard error for experiments with the human hand ranges from 0.6%-4.8%.

CRIS 6615-32000-031-00D

BIOCONTROL OF LESSER MEALWORM AND OTHER LITTER-DWELLING BEETLES IN POULTRY-REARING FACILITIES WITH BEETLBAR

D.A. Carlson and C.J. Geden

Objective: Use physical barriers to prevent migration of darkling beetle (lesser mealworm, LMW) larvae to avoid damage to facilities, to enhance control of houseflies in poultry-rearing facilities by managing appropriate populations, and to better manage manure. Use this tool to dramatically reduce the use of pesticides. We know that long-standing populations of LMW in chicken houses yield concomitant and unfortunate overproduction of larvae, and that the excess population of larvae leaves the litter, crawling up house posts into the cage area, some of which are eaten by the chickens. Before pupating, LMW larvae then burrow into new polystyrene and polyurethane thermal wall and roof insulation, destroying its effectiveness in a relatively short time. Other beetle larvae, such as the hide beetle, develop large populations and cause substantial damage to structural timbers, and are unaffected by treatment of the wooden posts by typical preservatives. This is a relatively new world-wide problem that has developed with the rise of large-scale poultry production.

Methods: Deployed BEETLBAR in two commercial poultry rearing facilities. Installed inexpensive, long-lasting physical barriers in poultry facilities, attached to each post, and to the inner walls of each house. Continued to develop ancillary devices to assist with non-chemical control of litter beetles.

Results: Collected data that showed 99.98 % effectiveness in preventing migrating larvae from reaching susceptible insulation, timbers and posts. Collected data to prove reduction of damage to facilities, reduction of pesticide use, contact between filth-breeding LMW that carry enteric diseases, together with enhancement of fly control. Further developed physical factors of BEETLBAR by maximizing effects while minimizing costs. Provided a practical demonstration of the application, and the robust character of this material and its cost effectiveness.

CRIS 6615-32000-031-00D

PEPTIDE HORMONE MIMICS OF TRYPSIN-MODULATING OOSTATIC FACTOR

D.A. Carlson and L. Okedi

Objective: Develop new species-specific methods of insect control based on peptide hormones that use natural, biorational routes to interfere with egg development, particularly of blood-feeding insects. Discover novel control methodology for blood-feeding insects near cattle-rearing facilities where integrated methods may be used, particularly an autocidal technique.

Methods: Synthetic peptides derived from *Aedes aegypti* (Diptera:Culicidae) Trypsin Modulating Oostatic Factors (TMOFs) were examined for their ability to inhibit vitellogenic growth in newly emerged females of *Stomoxys calcitrans* (Diptera: Muscidae). Single treatments by injection, or topical routes were applied to two day old females and they were fed on blood daily for five days.

Results: Some peptides arrested egg development when compared to control females seven days after treatment. The presence of underdeveloped ovaries in treated females was correlated with persistence of undigested blood-meals in the gut of such insects.

A series of synthetic *Aedes aegypti* Trypsin Modulating Oostatic Factor (AeTMOF) were evaluated for their ability to influence vitellogenic growth in 3-5 day old *Aedes aegypti* adult female mosquitoes. Some injection treatments of nine analogs retarded yolk deposition into ultimate egg follicles. Dose response to treatment was correlated to reduced blood protein digestion in the midgut of treated mosquitoes.

CRIS 6615-32000-031-00D

EVALUATION OF AMERICAN BIOPHYSICS' COUNTERFLOW 2000™MOSQUITO TRAP

D.L. Kline

Objective: The overall objective of this research thrust is to utilize traps as an alternate method of mosquito control. The specific objective of this project was to evaluate the efficacy of a new design of mosquito trap which utilizes patent pending counterflow technology in conjunction with a carbon dioxide attractant generator and thermoelectric power generator (fueled by propane gas) for stand alone operation. This trap is designed to be highly specific for biting flies thus providing an environmentally sound approach to reducing pesticide use while removing the target species.

Methods: Two models of the trap were evaluated during the course of this study. The main difference was a change in air flow profiles between Model 1 and Model 2 with the goal of improving trapping efficiency. The traps were evaluated in a large outdoor screened cage. One thousand (250 in each corner) laboratory reared, 4-6 day old, females of *Aedes aegypti* and/or *Ae. taeniorhynchus* were released ca. 2 hrs before sunset. Traps were operated continuously until 2 hrs after sunrise the next day, at which time the collections were brought into the laboratory for counting. In some trials 1-octen-3-ol (octenol) was used as an additional attractant. The Model 1 trap was operated in a wooded wetland site for 13 nights in 1997; Model 2 was operated at the same site for 11 nights in 1998. Model 2 was also field tested at Sea Island, Georgia, against a natural population of *Ae. taeniorhynchus* in May 1998.

Results: Model 1 was operated for four nights without octenol with *Ae. taeniorhynchus* as the target species and for 2 nights with *Ae. aegypti* as the target species. An average of 362.7 and 543.0 mosquitoes/night were captured for *Ae. taeniorhynchus* and *Ae. aegypti*, respectively. Model was operated with the addition of octenol for 8 more nights for *Ae. taeniorhynchus* and 5 more nights for *Ae. aegypti*, which resulted in mean collections of 508.0 and 298.6 per night for the two species, respectively. Thus, the addition of octenol seemed to increase the collection of *Ae. taeniorhynchus*, but decrease the collection of *Ae. aegypti*. Model 2 was operated for 4 nights for both species without octenol. This resulted in a mean capture rate/night of 637.7 for *Ae. taeniorhynchus* and 666.0 for *Ae. aegypti*. The Model 2 field test at Sea Island, Georgia, against a natural population of *Ae. taeniorhynchus* resulted in an average collection of 1230 mosquitoes/night for this species. In addition to mosquitoes the trap also caught an average of 9,127 biting midges/night. In 1997 at the wooded wetland site the Model 1 captured 134.3 mosquitoes/night. The tests in 1998 at the same site with Model 2 resulted in 133.6 mosquitoes/night. Nineteen different species of mosquitoes were caught in these traps at this site. Genera of mosquitoes collected included *Anopheles*, *Aedes*, *Coquillettidia*, *Culex*, *Culiseta* and *Psorophora*. In addition several species of *Culicoides* biting midges, *Lutzomyia* sandflies and *Diachlorus ferrugatus* tabanid flies were caught in both models. Very few non-biting nontarget specimens were caught. The Model 2 trap is now commercially available.

CRIS 6615-32000-031-00D

STABLE FLY TRYPSIN INHIBITORY FACTOR

L.M. Okedi and D.A. Carlson

Objectives: Develop new species-specific methods of insect control based on peptide hormones. The discovery of AeTMOF, the peptide hormone obtained from mature mosquito oocytes, gave rise to the search for a similar material in *Stomoxys calcitrans*, the stable fly (SF). Discover novel control methodology for blood-feeding insects near cattle-rearing facilities where integrated methods may be used, particularly an autocidal technique.

Methods: Stable fly and mosquito pupae were obtained from the insectaries. Upon emergence the adult stable flies were provided with sugar cubes and blood soaked cotton balls ad lib. Adult mosquitoes were provided with sugar cubes, wet cotton balls and guinea pig blood ad lib.
Extraction of Stable fly Ovary Derived Oostatic Factor: Stable fly ovaries were dissected and placed into 1mM PMSF, pH 4.5 over ice and homogenized. The homogenate was centrifuged at 20,000g for 20 minutes in the cold and the supernatant heat treated at 50°C for 10 minutes prior to lyophilizing.

Extracts prepared from vitellogenic ovaries of SF were assayed for trypsin modulating and oostatic activity in the stable fly and the mosquito, *Aedes aegypti*.

High Performance Liquid Chromatography (HPLC) and Matrix Assisted Laser Desorption Ionization-Time Of Flight-Mass Spectrometry (MALDI-TOF-MS) suggested that the SF ovary derived extract may be a peptide. Female specific peptides in vitellogenic females were not present in newly emerged, two day old females or older males.

Bioassays: Sep-Pak fractions were simultaneously screened for oostatic activity, as reduced lengths (um) of ultimate follicles over time, and Trypsin modulating activity, as nmol of BapNA/ minute/gut protein. The lyophilate was reconstituted with 0.1% Trifluoroacetic acid (TFA) and eluted on C18 reverse phase Sep-Paks with increasing concentrations of acetonitrile in 0.1% TFA as 10%, 20%, and to 50%. Various fractions were chromatographed on a C18 reverse phase HPLC and analyzed by MALDI-TOF-MS.

Results: Comparisons were done with the extract, and its fractions, with synthetic AedesTMOF and other proline rich analogs. The extract showed the presence of several low molecular weight peptides in oocytes from mature females that were not present in oocyte extracts from younger females or males. MALDI-TOF showed the presence of a candidate peptide at m/z 908.8in the bioactive fraction. This material was not present on corresponding fractions of other SF biological material. This experiment was repeated one year later with the same results. The SF-derived extract and fractions showed trypsin-modulating activity in the SF, but curiously, not oostatic activity. However, it showed both oostatic and trypsin-modulating activity in *Ae. aegypti*. This suggests that there may be another TMOF-like factor present in mature SF oocytes.

CRIS 6615-3200-032-00D

PRELIMINARY FIELD TESTS WITH THE MOSQUITO PARASITIC NEMATODE *Strelkovimermis spiculatus*

T. Fukuda, O.R. Willis and D.R. Barnard

Objective: To determine if the mosquito parasitic nematode, *Strelkovimermis spiculatus* would establish and recycle in mud-bottom and sod-lined concrete potholes containing water of various qualities.

Methods: Ten 5' x 6' concrete potholes were utilized. Eight were lined with sod and 2 with mud from a settling pond located at the University of Florida Swine Unit. Two mud-bottom pot holes and 2 sod-lined pot holes were filled with highly organic polluted water from the same settling pond. Two potholes were filled with partially polluted water (½ settling pond water and ½ well water) and the remaining potholes were filled with well water. *Strelkovimermis spiculatus* was introduced into the potholes six times during the period from September 15 to November 3, 1997. The nematode was introduced in two ways: (1) approximately 3,000 infected 4th instars of *Culex quinquefasciatus* were added to the potholes and the nematodes were allowed to emerge; (2) approximately 3,000 2nd instars of *Cx. quinquefasciatus* and approximately 24,000 newly hatched preparasites of *S. spiculatus* were added to the potholes and infection allowed to take place in the potholes. Since natural populations of mosquitoes were minimal, second instar *Cx. quinquefasciatus* were added to the treated potholes weekly, when available, for 22 weeks after the last treatment of November 3. Four days after the addition of larvae, a sample of at least twenty larvae were collected from each pothole, brought into the laboratory and the larvae were reared to the 4th instar, examined for nematode infection and percent infection calculated.

Results: Overall, the potholes in which the *S. spiculatus* was introduced with infected 4th instar larvae produced higher infection levels than the others, in which the infections were allowed to take place in the potholes. Infected larvae were first observed in the partially polluted and fresh water sod-lined potholes 4 weeks after the final treatment (11 weeks after initial treatment), with infection levels of 8.5 and 22.5%, respectively. Partially polluted potholes produced infected larvae in every collection from week 4 to week 22, except for week 2, with the highest level of infection at week 16 reaching 90.2% and finally dropping to 40.3% at week 22. Well water potholes produced infected larvae continuously during the 18 week period, with the highest infection level again at week 16 reaching 85.3% and a final level of 8.7% after the 22 weeks. The sod-lined polluted potholes produced infected larvae in the 5th post-treatment week and every week thereafter, with an initial level of 11.4%, a high at week 20 of 86.9% and a reduction to 19.2% at 22 weeks. Only 2, of the 22 weekly collections, week 17($\geq$1%) and week 20 (6.3%), from the mud-lined polluted potholes produced nematode infected larvae. This data indicates that *S. spiculatus* can become established and recycle in mosquito populations in organically polluted water.

CRIS 6615-32000-032-00D

EFFECT OF MANURE MOISTURE ON SEARCHING BEHAVIOR OF FIVE SPECIES OF HOUSE FLY PARASITOIDS

C.J. Geden

Objective: A more thorough understanding of niche partitioning by the various fly parasitoids could lead to improved matching of species for releases based on the ecological conditions of the microhabitats found in the target sites. Surprisingly little is known about preferences of the most common pteromalid species for different fly breeding substrates and for different environmental conditions within those substrates. The objective of the present study was to determine the effect of moisture levels of poultry manure on the foraging behavior of five species of fly parasitoids under conditions of high and low host availability.

Methods: Five species of pteromalid parasitoids were tested: *Muscidifurax raptor* (from Florida), *Spalangia cameroni* (Florida), *Spalangia endius* (Florida), *Spalangia gemina* (from Brazil), *and Dhirinus himalayanus* (from Morocco). Fresh (<24-h-old) poultry manure was collected and frozen to kill any arthropods present, partially dried in an oven, then reconstituted with water to 45, 55, 65, 75, and 85% moisture. Plastic dishes containing 120 cm^3 of manure were placed in grid patterns in large plexiglas boxes. House fly puparia (48-h-old) were then placed on the manure surface. Ten pupae/dish were placed in one assay box and 200 pupae/dish in a second box to provide two host:parasitoid ratios (2.5 or 50 pupae per parasitoid) for each experiment. Sixty female parasitoids were then introduced into each box. Pupae were removed after 24 h and held for fly and parasitoid emergence. Control pupal mortality was determined by placing bags of 100 pupae on manure dishes in a smaller box with no parasitoids.

Results: *M. raptor* showed a strong preference for dry manure when hosts were abundant, attacking and reproducing on nearly twice as many pupae in the 45% moisture manure as in the next wetter level. Substantial parasitism was observed up through the 75% moisture level, but very few pupae were attacked or parasitized in the 85% moisture manure. *Spalangia cameroni* attacked 30-40% of host pupae and produced 27-40 progeny from pupae in manure containing 45-65% moisture when hosts were abundant; few pupae were attacked or parasitized in manure with $\geq$ 75% moisture. *S. endius* showed a strong preference for dry manure, attacking and parasitizing twice as many pupae in the 45% moisture manure as in the 55% moisture level: few pupae were attacked or parasitized in manure with $\geq$ 75% moisture. *Spalangia gemina* showed the strongest response to pupae in the 55% moisture treatments, and activity was high in the 45 and 65% treatments as well. *D. himalayanus* foraged over the widest range of manure moisture of the five species studied, but overall rates of attack and progeny production for this species were very low. All of the *Spalangia* species showed a greater tolerance for high manure moisture levels when hosts were in short supply. A manuscript on this work has been submitted for publication in Environmental Entomology.

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EFFECT OF AIR FLOW ON HOUSE FLY DISTRIBUTION PATTERNS IN CAGED-LAYER POULTRY HOUSES

C.J. Geden, J.A. Hogsette, and R.D. Jacobs

Objective: The ecological factors affecting distribution patterns of adult house flies (*Musca domestica* L.) in animal confinement facilities are, for the most part, unknown. Modern poultry houses are being designed to maximize the rate of air movement through the facilities to improve bird health and performance. The objectives of this study were to determine whether airflow influenced the orientation and distribution of house flies in closed poultry houses, and to determine whether there is a correlation between fly counts using spot cards and sticky cards.

Methods: Studies were conducted at two poultry facilities in Florida representative of evaporative cooling and tunnel ventilation systems. Spot cards (which measure relative fly abundance) were placed on either the upwind or downwind sides of building support posts to assess the effect of air movement on fly distribution. Cards were replaced weekly for one year in one study and for four weeks a second study. Fly fecal and vomit spots on the cards were counted as a measure of fly activity. In addition, the relative sensitivity of spot cards were compared with another fly monitoring tool, the sticky card.

Results: Numbers of fecal and vomit spots deposited by house flies on spot cards were about twice as high on cards placed on the downwind sides as on the upwind sides of building support posts in poultry houses in Brooksville, FL. This trend was stronger at the ends of the houses, where airflow is greater, than in the relatively still-air center of the houses. In a similar evaluation conducted in a pullet house in Zephyrhills, FL, significantly higher fly counts were observed on both spot cards and sticky cards in downwind than in upwind orientations. Flies in the pullet houses were concentrated in both ends of the house and in the center region, with comparatively fewer flies in the intermediate zones. There was a high degree of correlation between spot card and sticky card counts. These results may allow greater precision in the targeting of behavior-based adult fly control technologies such as light traps and bait stations. A manuscript on this work has been submitted for publication in the Journal of Economic Entomology.

CRIS 6615-32000-032-00D

ARRIVAL PATTERNS OF SPRING POPULATIONS OF STABLE FLIES IN EASTERN KANSAS.

J.A. Hogsette and A.B. Broce.

Objective: The method of repopulation of the plains states with stable flies, *Stomoxys calcitrans,* in the spring has been discussed for some time, but is still largely unknown. Ranchers talk of swarms of stable flies appearing suddenly after rains in early late May or early June, however synchronized eclosion is unknown in higher Diptera, and large populations of overwintering adults have never been found in the regions in question. Therefore we attempted to determine if stable flies were moving into areas of eastern Kansas with large scale weather systems.

Methods: Working with Dr. A. B. Broce, Kansas St. Univ., Manhattan, cooperators were found on ranches and farms in eastern Kansas from the Nebraska border to the Oklahoma border who would monitor stable fly traps placed on their property. The optically attractive traps were covered with clear sticky sleeves on which flies were trapped. Cooperators changed the sticky sleeves twice weekly and sent them to KSU in stamped, addressed envelopes we provided. Flies were counted and data compared and correlated with weather features such as barometric pressure and wind speed and direction. Flies were also analyzed for presence of blood meals as an indication of age and feeding activity.

Results: This is the second year of our study and weather activity was more typical this year. Fly population increases appear to be closely related to decreases in barometric pressure and changes in wind speed and direction that signal the passage of large-scale synoptic systems, such as cold front. Flies caught at the beginning of the study had taken blood meals, but were still physiologically young. This indicated that flies emerged, and fed at a distant location, then moved on to the capture sites. These flies had not yet produced eggs. Flies captured later were similar, but these flies had not yet fed on blood. This indicated that flies captured later in the study were produced locally. These data are similar to those we collected in western Kansas in 1996, and indicate that stable flies are repopulating Kansas from distant sites with later assistance from local populations.

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DETERMINATION OF THE ABILITY OF BLACK DUMP FLY, *Hydrotaea aenescens,* LARVAE TO DEVELOP IN THE MANURE OF ADULT CATTLE.

J.A. Hogsette and R. Farkas.

Objective: Black dump flies have been found to reproduce and develop in the manure of unweaned calves, but nothing is known of their ability to utilize the manure of adult, lactating dairy cattle. To determine whether black dump flies have biological control potential for nuisance fly control around adult dairy cattle, development of black dump flies in manures of adult dairy cattle was determined.

Methods: In Budapest and Gainesville, manure samples were collected from adult lactating dairy cattle as soon as manure was dropped onto the ground. Manure was containerized, returned to the laboratory and frozen to kill any unwanted organisms. When ready for testing, manure was allowed to thaw to room temperature, portioned into 200-ml cups and weighed. First-instar larvae of *Hydrotaea aenescens* were placed on manure, and on similar cups of standard laboratory diet, which were used as untreated controls. Cups were monitored for development, and pupae were weighed and held for adult eclosion.

Results: *Hydrotaea aenescens* was able to develop successfully in manure from adult lactating dairy animals. Numbers of pupae and adults and pupal weights were not significantly different from those of *H. aenescens* developing in manures produced by calves aged 3 to 8 weeks. This was a surprising result because immatures of *H. aenescens* have rarely if ever been reported from dairy manures and it has been assumed that *H. aenescens* was unable to use cow manures for development of the immature stages. Data have been pooled with data from calf manure studies and the resulting manuscript is in in-house review.

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COMPARISON OF *Edhazardia aedis* INFECTION IN ITS NATURAL HOST *Aedes aegypti* AND AN UNNATURAL HOST *Aedes albopictus*

M.A. Johnson, J.J. Becnel and A.H. Undeen

Objective: To examine the process of infection of the pathogenic microsporidium *Edhazardia aedis* in its natural host, *A. aegypti* and an unnatural host, *A. albopictus*.

Methods: Four laboratory experiments were performed to analyze and compare the infection of *E. aedis* in both hosts. In each experiment, larvae of *A. aegypti* and *A. albopictus* were exposed to spores of *E. aedis* and the following parameters assessed: 1. Percent germination of spores *in vivo* at one hour postexposure. 2. Initial site of infection and early development was identified and recorded by preparing larval and pupal mosquitoes for light and electron microscopy at 24 h intervals postexposure. Adults were processed shortly after emergence, one week after emergence, and before and after taking a blood meal. Slides and thin sections of larvae, pupae and adults were examined and the developmental stages of *E. aedis* and site of infection were recorded. 3. Transovarial transmission in both species was determined by blooding and egging female adults. Each female and her eggs were smeared, stained, and examined for presence of *E. aedis*. 4. Loss of infection was assessed as the larvae pupated, underwent metamorphosis, and emerged as adults. Fourth instar larvae and adults were smeared, stained and percent infection was recorded.

Results: Results of the *in vivo* germination test demonstrated that *E. aedis* spores germinated successfully in both species of mosquitoes. The percentage germination of spores in *A. aegypti* guts (88.4 ± 1.6, mean ± SE) did not differ significantly from *A. albopictus* (81.8 ± 1.6, Mean ± SE) ($p<0.01$, $F=8.56$, $n=100$). The initial site of infection was the gastric caeca in both *A. aegypti* and *A. albopictus*, however, different areas of this organ were infected in each species. In *A. aegypti,* large numbers of developmental stages and first binucleate spores were found in the cells of the gastric caeca. In *A. albopictus,* the initial sites of infection were the tracheal epithelial cells surrounding the gastric caeca. In addition, these areas were heavily melanized showing intense host immune reaction. Transovarial transmission never occurred in *A. albopictus*. A mean of 75.3 ± 8.2% of *A. aegypti* eggs were found containing sporoplasms of *E. aedis*. None of the *A. albopictus* eggs examined contained any stages of *E. aedis*. *Aedes albopictus* appeared to lose *E. aedis* infection as the larvae passed through the pupal stage while *A. aegypti* remained infected before and after metamorphosis. Before the onset of pupation, the percent of larvae infected of both mosquito species was 100% as revealed by Giemsa stains. After adult emergence, the percent of *A. aegypti* infected, as revealed by Giemsa stains and presence of second binucleate (transovarial) spores, remained at 100%. The percent infection in adult *A. albopictus* dropped to 72% (Giemsa-stains) with only 23% with second binucleate spores responsible for transovarial transmission. Light microscopy observations of fresh tissue from adults also revealed fewer second binucleate spores in *A. albopictus* than in *A. aegypti*.

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MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF THE Thelohania solenopsae COMPLEX (MICROSPORIDIA: THELOHANIIDAE)

B.A. Moser, J.J. Becnel, and D.F. Williams

Objective: To determine the relationship of Thelohania sp. isolated from the red imported fire ant Solenopsis invicta in Florida to T. solenopsae (type species) from S. invicta (type host) in Brazil and Thelohania sp. from the black imported fire ant S. richteri in Argentina.

Methods: Recently, a microsporidium has been discovered in S. invicta in Florida that is indistuinguishable from T. solenopsae (Brazil) and Thelohania sp. (Argentina) by comparison of stages with the light microscope. Spore ultrastructure and the 16S rRNA gene sequence of this isolate were determined for comparative purposes to T. solenopsae (Brazil) and Thelohania sp. (Argentina). Infected workers and male and female alates of S. invicta were collected near Gainesville, Florida, USA. Microsporidian spores were prepared for transmission electron microscopy to determine spore ultrastructures. Crude DNA extracts of spores isolated from 3 colonies were prepared for amplification of the 16S rRNA gene. Pooled gene product from three separate PCR reactions for each of the 3 colonies was sequenced directly. The sequences were completed by redundant sequencing of both strands. Ultrastructural features and 16S rRNA gene sequence were compared to those of the microsporidia isolated from fire ants in Brazil and Argentina.

Results: Meiospores and free spores of T. solenopsae (Brazil), Thelohania sp. (US) and Thelohania sp. (Argentina) had similar features and could not be differentiated by ultrastructural comparison. The sequences of Thelohania sp. (US) from the 3 colonies were identical, and the complete 16S rRNA gene was 1383 nucleotides long with a GC content of 41%. The sequence of Thelohania sp. (US) differed by 11 nucleotides from T. solenopsae (Brazil) and by 12 nucleotides from Thelohania sp. (Argentina). These data in conjuction with light-microscopic and ultrastructural evidence suggest that the Floridian Thelohania sp. is closely related to T. solenopsae (Brazil) and Thelohania sp. (Argentina). Because these three Thelohania isolates are difficult to distinguish, we suggest to group them into a T. solenopsae complex. The Thelohania solenopsae complex in this study is composed of 3 different isolates: Thelohania solenopsae from S. invicta (type species and host) (Brazil), Thelohania sp. from S. invicta (US)., and Thelohania sp. from S. richteri (Argentina) Molecular and morphological data have provided important information in the characterization of the members of the T. solenopsae complex, yet we feel that the question of conspecificity is still not resolved satisfactorily. The main reason for this is that to date cross-transmission and infectivity studies have not been successful even though important progress has been made in this area (unpublished observations). Until the question of conspecificity is resolved unambiguously, we propose to refrain from collectively naming all the different isolates T. solenopsae. Instead, we suggest that the different isolates be referred to as: T. solenopsae (Brazil), T. solenopsae (US), and T. solenopsae (Argentina).

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MOLECULAR CHARACTERIZATION OF A MOSQUITO PATHOGENIC VIRUS FROM MOSQUITOES PRODUCED IN AGRICULTURAL WASTEWATER

B.A. Moser, A.F. Cockburn, and J.J. Becnel

Objective: To characterize the genome of a novel mosquito-pathogenic virus.

Methods: Viral occlusion bodies were purified from infected larval mosquitoes by differential centrifugation on a Ludox gradient. To quantify occlusion body concentrations spectrophotometrically, a standard curve at OD260 was established. Occlusion body concentrations of samples that were used for OD260 readings were determined by counting using darkfield microscopy optics. Viral DNA was extracted and cut with several restriction endonucleases to obtain a size estimate of the viral genome and to identify suitable enzymes for construction of a genomic library. In addition, the size of the viral genome was estimated by pulsed-field gel electrophoresis. For sequencing of the whole viral genome, viral DNA was restricted, cloned and sequenced.

Results: Density gradient centrifugation of infected material on a continuous Ludox gradient, commonly used to isolate microsporidia, was adapted to efficiently purify viral occlusion bodies. After centrifugation was completed, the occlusion body band was washed in 0.0001 N NaOH, pH 10.0 to prevent crystallization of and to remove the silica. The occlusion bodies had a density of 1.14-1.18g/ml. We determined that an OD of 1 corresponds to approximately 4.5x107 occlusion bodies/ml. Based on restriction enzyme analysis and pulsed-field gel electrophoresis, the genome of the mosquito-pathogenic virus was approximately 75 kbp. The sequencing of the whole genome is in progress.

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MUTANTS OF *Aedes aegypti*

J.A. Seawright

Objective: To induce dominant mutations, with emphasis on non-biting types, in *Aedes aegypti*.

Methods: Males, < 24 hours old, were irradiated with 3.5 Kr in a radioactive Cesium source. These males were crossed to untreated females, and the F_1 progeny were screened for dominant mutants, both morphological and non-biter types. The larvae, pupae, and adults were examined with a stereo microscope for the presence of morphological mutations. Crosses were done to ascertain the inheritance of the morphological mutants. The F_1 females were offered a blood meal on bovine blood held in a collagen membrane; if the females were not able to feed through the collagen, then they were offered a blood meal on guinea pigs. Any female that could not bite was offered a blood meal of bovine blood on a cotton pad. Eggs were collected from those that could feed, and their progeny were then assessed for biting capability.

Results: Approximately 20,000 mosquitoes were examined. Several morphological mutants were recovered: (1) *orange body* - dominant, autosomal, larvae with a distinct body color, (2) *melanotic* - dominant, sex-linked, some lethality in homozygotes, larvae with very dark pigmentation, (3) *clear cuticle* - recessive, autosomal, probably the same as yellow larva which was described previously, (4) *black setae* - no inheritance data yet. The inheritance patterns and assignment to positions on the genetic map are underway. There were several other mutants that were either lethal or sterile, viz. (1) *singed setae* - larvae with underdeveloped setae observed 4 times (3 lethal and 1 sterile), (2) *long anal papillae* - observed in one specimen (sterile), (3) *colorless* - larvae with no pigmentation except for the eyes - observed 3 specimens (lethal). Ten non-biter types were observed. These females could feed on 10% sugar water, and they tried unsuccessfully to penetrate the collagen and guinea pig; only 3 were able to feed on blood on a cotton pad. The inheritance of the non-biter types appeared to be complicated, and no truly useful mutant was isolated.

POSTHARVEST

AND

BIOREGULATION

CRIS - 6615-43000-007-00D--Population Management of Insects to Protect Stored Products

CRIS - 6615-43000-008-00D--Detection and Population Estimation of Stored Product Insects

CRIS 6615-43000-007-00D

THE EFFECTS OF JUVENOID AGONISTS ON THE PHYSIOLOGY OF *PLODIA INTERPUNCTELLA* AND *GALLERIA MELLONELLA*

S. Dyby and D. Silhacek

Objective: To determine the physiological basis of juvenoid agonist toxicity during embryogenesis and larval development of the Indian meal moth.

Methods: Last instar larvae or larvae ready to molt to the last instar were injected with LPA complexed with BSA, bombesin/BSA, juvenile hormone I /BSA, or BSA in an aqueous buffer. Concentrations of injected material ranged from 0.5 µg/ml to 100 µg/ml. Diet laced with LPA or bombesin was prepared by using 1 mg/ml LPA or bombesin complexed with BSA in buffer, diluting it in honey-glycerol, and mixing this with the dry diet component to reach a specified concentration, from 5 ppm to 100 ppm. A similar approach was used for juvenile hormone I, fenoxycarb, oleic acid, and BSA in buffer.

Results: Lysophosphatidic acid (LPA) and bombesin are growth factor-like compounds that have manifold effects on cell shape and metabolism. LPA and bombesin specifically activate Rho, a small GTP binding protein that is part of a signalling pathway that activates early response genes such as c-*fos,* which in turn regulate cell growth.

Injecting LPA in Plodia interpunctella embryos or larvae produces a set of developmental defects like those caused by excess juvenile hormone or by juvenoid agonists. When Plodia interpunctella larvae are fed diet laced with LPA or bombesin, similar defects occur. We have established a dose-response pattern in Plodia interpunctella for LPA and bombesin using three means of contact: diet, injections, and immersion. A comparable set was done with juvenile hormone I and fenoxycarb, as well as oleic acid and BSA in buffer. Injecting LPA into last instar Galleria mellonella larvae also produces larval-pupal intermediates and curtails full evagination of imaginal discs, giving rise to defective legs, wings, and mouthparts.

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JUVENILE HORMONE AND JUVENILE HORMONE MIMICS INHIBIT CELL DIVISION IN AN IMAGINAL DISC CELL LINE

H. Oberlander, C.E. Leach, and E. Shaaya[1]

Objective: To evaluate an imaginal disc-derived cell line as a bioassay tool for juvenile hormone and juvenile hormone mimics.

Methods: The Indianmeal moth cell line established from wing imaginal discs by Lynn and Oberlander has been utilized for investigations of ecdysteroids, and recently for assays with juvenile hormone mimics. The cells are maintained in antibiotic-free Grace's medium supplemented with 10% heat-inactivated fetal bovine serum. New subcultures were prepared and maintained in culture flasks at 26 °C. for 72 hours. Test compounds were dissolved in acetone and then absorbed onto fatty acid-free bovine serum albumin. The acetone was then removed and the test compounds were dissolved in Grace's medium. The culture medium in test flasks was then replaced with new medium containing the test compounds and followed over an additional 3 days. The cell number was assessed by direct counts of the monolayer cultures using a grid system on the flask with the aid of an inverted phase microscope.

Results: Control experiments with linoleic acid at 10, 50 and 100 ppm had no effect on the cell numbers in the cultures during the 3 day test period. However, Juvenile Hormone I, fenoxycarb, and farnesol at 50 or 100 ppm prevented any increase in cell number over the test period. Juvenile hormone II suppressed proliferation only at 100 ppm, while methoprene inhibited proliferation at 75 ppm or higher. These results indicate that this cell line may be useful for studies on the mode of action and receptors for juvenile hormone. However, it is clear there is not a complete correspondence between activity in vitro and in vivo, since we would not have expected farnesol to have higher activity in this assay than methoprene, for example. Additional research is being conducted in other laboratories to determine whether this cell line will be as useful for juvenile hormone receptor studies as it has been for ecdysteroid investigations.

1. Prof. Eli Shaaya, Volcani Center, Bet Dagan, Israel

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REVERSION OF THE TRYPTOPHAN OXYGENASE MUTATION OF THE MOTH, *ANAGASTA KÜHNIELLA A* STRAIN IS INDUCED BY THE *PIGGYBAC* TRANSPOSASE

O.P. Perera and P.D. Shirk

Objective: To determine the utility of the *piggyBac* transposon as a gene vector in Lepidoptera. The yellow eye color phenotype of the *Anagasta kühniella a* strain is the result of a mutation in the locus that produces the tryptophan oxygenase gene. The availability of cDNA clones for the wild type tryptophan oxygenase gene from other insects provides a clear potential for utilizing this mutation during germ line transformation as a marker for movement of a transposon vector. These experiments were to test the suitability of the tryptophan oxygenase mutation of *A. kühniella a* strain as a means of detecting the movement of the *piggyBac* transposon in this moth.

Methods: The tryptophan oxygenase (TO) from *Anopheles gambiae* under the control of the IE1 promoter (provided courtesy of N. J. Besansky, U. Notre Dame) was inserted into the *piggyBac* transposon. The *piggyBac/IE1-TO* construct was co-microinjected with a *piggyBac/wc-hsp70-transposase* helper into preblastoderm embryos of the *A. kühniella a* strain.

Results: From 1504 microinjected eggs, 241 adults emerged and were mated with *A. kühniella a* strain adults. From those matings, 111 fertile matings occurred and 5 of those matings resulted in progeny with a brown eye phenotype suggesting the presence of an active tryptophan oxygenase. Three of these brown eyed families produced progeny and are being selected for homozygous breeding of the phenotype. Analysis of the genomic DNA from each of these families has shown that the *piggyBac/IE1-TO* is not present in these families. In the control experiment where the *piggyBac/wc-hsp70-transposase* helper alone was microinjected into 1200 eggs, 2 matings occurred where the brown eye phenotype was observed in the progeny. These findings suggest that the induction of the brown eye phenotype occurs because of the presence of the *piggyBac* transposase. This would also suggest that the mutation of the tryptophan oxygenase in the *A. kühniella a* strain is the result of the insertion of a TTAA type transposon that can be cross mobilized by the *piggyBac* transposase.

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THE POTENTIAL OF INSECT GROWTH REGULATOR APPLICATIONS FOR MANAGING MOTH POPULATIONS IN PACKAGED COMMODITIES

D.L. Silhacek, S. Dyby and C. Murphy

Objective: To determine if treatments with juvenoid agonists (JH_{Ag}), ecdysteroid agonists or chitin synthesis inhibitors can effectively protect commodities from insect damage during storage.

Methods: We previously reported the results of laboratory tests on the Indian meal moth, Plodia interpunctella, that showed adult females laid non-viable eggs following a six-hour or longer exposure to surfaces treated with a JH_{Ag}. In our studies during the past year, we examined the efficacy of using fenoxycarb treatments to protect commodities stored in simulated warehouse conditions. In a typical test, small amounts of various commodities were confined in individual paper bags and taped. Each bagged commodity was placed in a 4"x4"x4" corrugated cardboard box and sealed with masking tape. Three boxes of seven different commodities were placed on each of three pallets arrayed in a 175 ft^2 simulated warehouse. Approximately 1,000 Indian-meal moths were allowed to emerge in this warehouse space to mate and lay eggs. Each test consisted of four similar-sized warehouses. A series of tests were conducted sequentially over a period of nine months to determine the longevity of the treatments under warehouse conditions.

The effectiveness of the JH_{Ag} treatments on two other flour moths, *Anagasta kuehniella* and *Cadra cautella*, as well as a wild strain of *P. interpunctella* were compared with the laboratory strain of *P. interpunctella*. For these tests, tempered masonite panels were treated with the JH_{Ag} and assembled into 6"x6"x6" boxes to confine and expose the moths to the treatment for 24 hours. The viability of eggs laid by treated moths was determined following treatment.

Results: We placed packaged commodities inside sealed cardboard boxes and stored them at one of four treatment conditions. When the commodities were exposed to high numbers of newly-emerged Indianmeal moths, there was no infestation of commodity when the warehouse walls and the outer-surface of the cardboard boxes were treated with 15µg fenoxycarb\cm^2 (condition 1); similar results were observed when only the warehouse walls were treated (condition 2). However, the treatment where only the cardboard boxes were treated (condition 3) showed moderate infestation.

In normal warehouse situations, the boxed commodity would contribute a much greater percentage of the vertical resting surfaces (favored by moths) making the commodity-only treatment somewhat more effective and the wall-only treatment somewhat less effective. We project that for effective warehouse applications, all vertical surfaces, in the warehouse and the commodity will have to be treated with an appropriate JH_{Ag}. The longevity of fenoxycarb in the warehouse has been found to be about six months. When no JH_{Ag} treatment was applied (condition 4), heavy infestations were observed---but the level of infestation was very commodity dependent.

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MONITORING STORED-PRODUCT INSECTS IN NATURAL PRODUCT WAREHOUSES

R.T. Arbogast, P.E. Kendra and R W. Mankin

Objective: A wide range of natural plant products, such as ginseng. St. John's wort, and saw palmetto, are used as dietary supplements. Plant parts are harvested, usually from their natural habitats, dried , and stored in warehouses until they are shipped to end processors. While in storage, they are subject to infestation by storage pests. In the spring of 1998, we had an opportunity to study an insect infestation of bagged saw palmetto berries, *Serenoa repens* (Bartram) Small, stored in a steel warehouse (30.5 by 15.2 m.) in central Florida. Our objective was to test the effectiveness of trapping and contour analysis of trap counts as a monitoring method to detect and locate foci of infestation and to evaluate the effectiveness of control intervention.

Methods: Moths were monitored with pheromone-baited sticky traps (SP-Locator traps with Minimoth lures, AgriSense, Mid Glamorgan, UK), and beetles with pitfall traps (FLĪT-TRAK M^2, TRÉCÉ, Salinas, California) baited with *L. serricorne* and *Tribolium* pheromones and a food attractant oil (furnished with the traps). A moth trap and beetle trap were placed at each of the locations indicated in Fig. 1. Trap location was specified in rectangular coordinates with the origin at one corner of the warehouse. Some moth traps were attached, by means of Velcro, to the walls of the warehouse, with the sticky surface oriented horizontally. Others were attached, to the tops of wooden stakes supported by stands on the floor or to bags on top of the stacks. They were located at heights ranging from 1.2-3.8 m. Beetle traps were placed either on the floor or on top of the stacks (0.0-3.4 m). Trap catch was recorded daily for 4 days while the berries were in the warehouse, and again for 4 days after the berries had been removed and the warehouse thoroughly cleaned. Contour analysis was done with Surfer Version 6.02 (Golden Software, Golden, Colorado).

Results: The infestation involved mainly five species of stored-product insects: *Plodia interpunctella* (Hübner), *Cadra cautella* (Walker), *Lasioderma serricorne* (Fabricius), *Tribolium castaneum* (Herbst), and *Oryzaephilus mercator* (Fauvel). Trapping and contour analysis effectively mapped the distribution of each pest species in the warehouse, and located foci of infestation, mostly in the stacks of bagged saw palmetto berries (Fig. 1). For example, *O. mercator* was limited to stacks S-2, S-3, and S-4, with the major center of infestation in S-4 (Fig. 1 A). *C. cautella* was widely distributed with major centers in stacks S-3 and S-5 and in an area, adjacent to the store room and rest room, used for storage of empty burlap bags and equipment (Fig. 1 B). Trap captures after warehouse cleanup were much less numerous and occurred mostly along the walls, which were covered with plywood. This suggested that the space enclosed between the plywood and the outer wall was harboring insects. The plywood was removed, revealing an accumulation of plant debris. This was removed, and the plywood was cut before replacement leaving an opening next to the floor to facilitate sanitation. We will trap again when the warehouse is full to see if the problem has been mitigated.

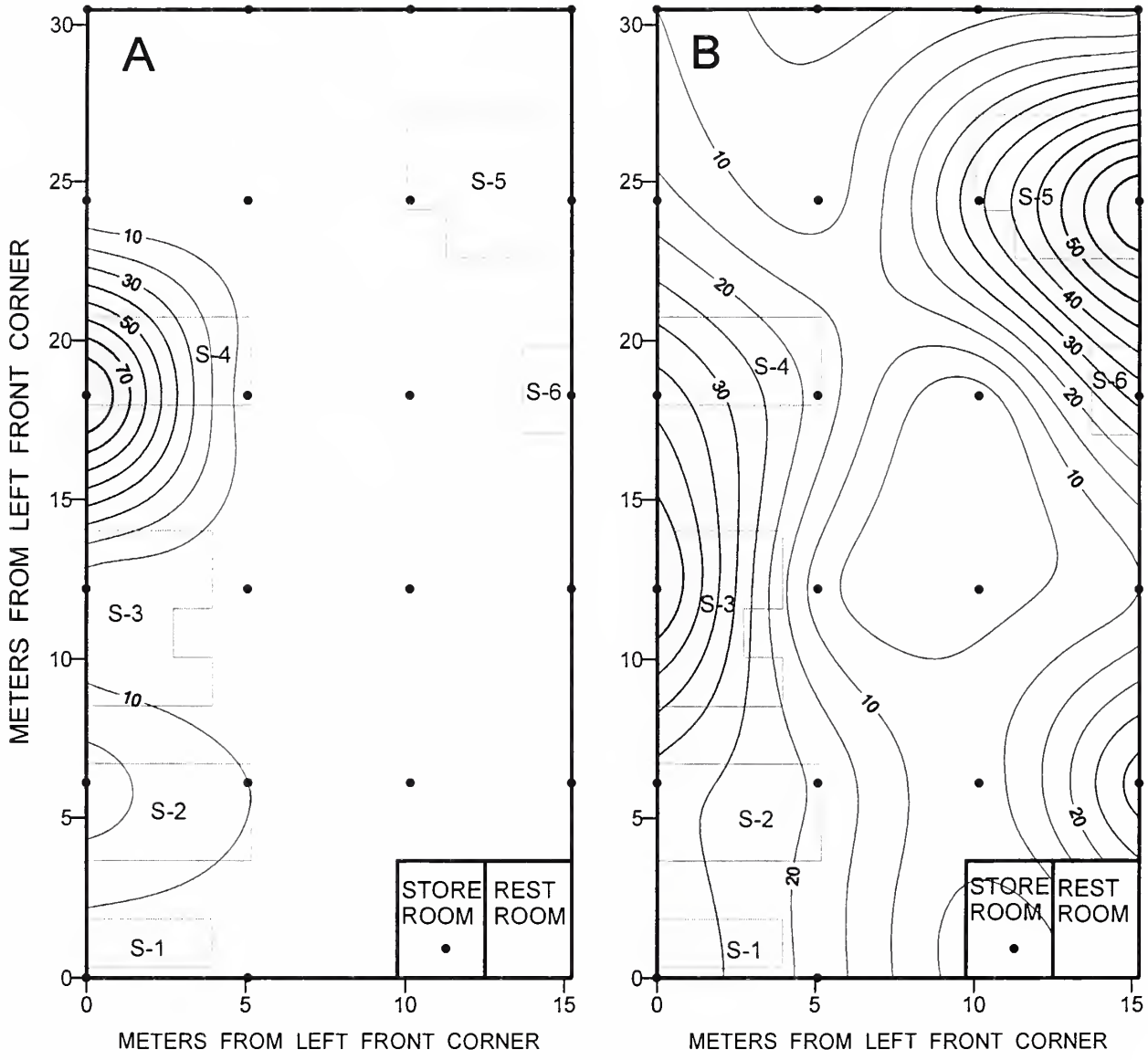

Figure 1. Spatial distribution of insects infesting bagged saw palmetto berries stored in a warehouse in central Florida. (A) *Oryzaephilus mercator* sampled with baited pitfall traps. (B) *Cadra cautella* sampled with pheromone-baited sticky traps. Solid dots indicate trap positions, and contour lines represent numbers of insects captured in a trap over a period of four days. Fine lines enclosing designations S-1, S-2, ... indicate stacks of bags.

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EVALUATION OF A MANUFACTURED ELECTRONIC GRAIN PROBE INSECT COUNTER (EGPIC)

N.D. Epsky and D. Shuman

Objective: The Electronic Grain Probe Insect Counter (EGPIC) is a commercial grain probe trap that has been modified by adding an infrared sensor head that electronically counts insects as they fall through. All components of the EGPIC system to date have been produced in-house at the USDA/ARS laboratory in Gainesville, Florida. There are several labor-intensive steps needed to produce the components for the electronic probes, but some are amenable to commercial manufacturing processes. The electronic probes are the critical components in determining the system's performance. Ability to precision mill the probe head and funnels using commercial manufacturing processes has allowed small-scale replication of the EGPIC system. Reported herein are tests of manufactured prototype EGPIC systems.

Methods: Replications of the EGPIC system were produced by Analytical Research Systems, Inc., Gainesville, FL. Tests were conducted to determine if the materials and manufacturing procedures used produced electronic probes that accurately counted the number of insects that were captured. Baseline accuracy of the manufactured probes was determined with drop tests using dead insects. Ten sets of ten insects were dropped through a probe, and number of insects counted was recorded. Accuracy was based on the electronic count per 100 insects. Rusty grain beetles, sawtoothed grain beetles and red flour beetles were used for these tests. Production specifications required > 85% accuracy in tests with the smallest insect, the rusty grain beetle, with accuracies > 90% preferred.

Results: Six EGPIC systems were produced and 54 electronic grain probes have been tested. Baseline accuracy ranged from 87 - 100% accuracy drop tests with rusty grain beetles, with an average accuracy of 93.4%. In tests with sawtoothed grain beetles, baseline accuracy ranged from 93 -100%, with an average accuracy of 98.3%. In tests with red flour beetles, baseline accuracy ranged from 97 -100%, with an average accuracy of 99.5%. Availability of manufactured EGPIC systems will aid in technology transfer of the EGPIC system by expanding its use in research, validating its potential as a stored-product pest management tool, and increasing its availability to the agricultural industry.

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ACTIVE SPACES OF PHEROMONE TRAPS FOR *PLODIA INTERPUNCTELLA* (LEPIDOPTERA: PYRALIDAE) IN ENCLOSED ENVIRONMENTS

R.W. Mankin, R.T. Arbogast, P.E. Kendra, and D.K. Weaver

Objective: Insect infestations of commodities in department stores and warehouses are usually clumped into small areas. *Plodia interpunctella* moths are the major pests in many infestations, and sex-pheromone-baited traps have been used to pinpoint infestations and localize treatment within affected areas. Some types of trap are better at pinpointing infestations than others. This study examined the characteristics of traps designed to have a short range in storage environments. Factors contributing to the short range were investigated to assist in interpretation of trap catches and develop improved designs.

Methods: Four combinations of two sticky traps and two lures emitting the *P. interpunctella* sex pheromone, (Z,E)–9,12–tetradecadien–1–ol acetate (ZETA), were tested in a wind tunnel [SP-Locator (AgriSense Ltd, Mid Glamorgan, UK) or Pherocon II (Trécé Inc., Salinas, CA) traps combined with Minimoth (AgriSense) or IMM+4 (Trécé) lures]. The SP-Locator traps with Minimoth lures were selected for further bioassays in a research shed because of their success in spatially targeting insect infestations in preliminary tests in department stores. Caged moths were set at different distances from traps in the shed and their wing-fanning and flight responses to pheromone were observed over 30-s intervals after lures were placed in the traps. The emission rates of 5 Minimoth lures (septa) were measured to enable comparisons of the measured attractive range with the predictions of pheromone dispersal models.

Results: Comparisons of the numbers of moths taking flight and numbers captured with different combinations of trap and lure types are shown in Table 1. The response to the Minimoth lure was lower than the response to the IMM+4 lure, probably because of a lower emission rate. In the research shed, males exhibited flight or wing–fanning responses at distances up to ~4 m from the traps, in good agreement with predictions of previously developed pheromone dispersal models. At all tested distances where moth responses were detectable, the responsiveness habituated rapidly (~3 min half–life). This behavioral habituation may be reproductively advantageous by conserving energy when a mate is not located quickly. The Minimoth lures emitted ZETA at ~2.3 ng/h, or 10% of typical rates for lures in the field. The low emission rate contributes to its observed capability to spatially target *P. interpunctella* infestations. The habituation response provides an ancillary benefit for spatial targeting of stored product insect infestations in department stores and warehouses by decreasing the attractive ranges of these traps from 4 m to 2–3 m within 5–10 min after the moth's initial exposure to pheromone.

Table 1. Numbers of male *P. interpunctella* taking flight in wind tunnel and numbers captured with different combinations of pheromone traps and lures

Trap	Lure	No.		
		Tested	Flying[a]	Captured[b]
Pherocon II	IMM+4	31	27	23
Pherocon II	Minilure	38	26	15
SP–Locator	Minilure	32	29	23
SP–Locator	IMM+4	33	30	28

[a] $\chi^2 = 9.10$, df = 3, $P < 0.05$
[b] $\chi^2 = 21.9$, df = 3, $P < 0.05$

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EGPIC WORKING GROUP

D. Shuman, N.D. Epsky, R.T. Arbogast, J.E. Throne, F.H. Arthur, C. Burks, D.K. Weaver, and T.W. Phillips

Objective: To aid in the technology transfer of the Electronic Grain Probe Insect Counter (EGPIC) System.

Methods: The Electronic Grain Probe Insect Counter (EGPIC - U.S. Patent No.5,646,404 issued 7/97) is a system developed to monitor infestations in stored-products. It uses infrared-beam sensors to electronically count the numbers of insects that crawl into and fall through perforated tubes distributed throughout stored agricultural products, and then displays and records these counts at a central computer. The Electronic Grain Probe Insect Counter (EGPIC) Training and Cooperative Research Workshop was held at CMAVE, Gainesville, FL in May 1998 to aid in technology transfer of the EGPIC System by expanding its use in research, field validating its potential as a stored-product pest management tool with a variety of commodities over a range of geographic locations, and increasing its exposure to the agricultural industry. The workshop was attended by scientists and technicians who would be using EGPIC Systems for their research, and by industry representatives who are interested in the potential of this technology. The agenda of the workshop was 1) to review development of the EGPIC System, 2) to conduct a training session of engineering and entomological aspects of EGPIC use, 3) to present individual research interests and objectives with EGPIC, and 4) to facilitate development of cooperative research projects.

Results: Analytical Research Systems, Inc., Gainesville, FL, manufactured replicates of the prototype EGPIC System that were then completely tested and characterized at CMAVE in order to document and insure their quality prior to shipping to participating work group members. All the EGPIC Systems have been delivered to the work group members. A field validation study is being conducted in corn and wheat in Kansas, wheat in Oklahoma and Montana, corn in Florida, and nuts in California. The count accuracy field data already obtained from both California and Oklahoma have been quite good, even during and after phosphine fumigations. Other research under way is 1) trap interpretation and utilization of the EGPIC time-stamped insect count data, 2) optimization of trap body design, 3) environmental, biological, and behavioral factors that might affect trap capture, and 4) differentiation of insect species based on body size. An article on EGPIC in the September, 1998 issue of Agricultural Research magazine published by ARS and a keynote speech describing EGPIC at the 7th International Working Conference on Stored-product Protection in Beijing, China in October, 1998 has provided exposure that has resulted in a large interest by the private sector in both the licensing/commercialization and the purchasing of EGPIC Systems. It is anticipated that EGPIC will become commercially available in the near future.

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DETECTION AND POPULATION ESTIMATION OF STORED PRODUCT INSECTS: EGPIC

D. Shuman and N.D. Epsky

Objective: To develop and evaluate automated systems for monitoring infestations in stored-products and for quantifying hidden infestations in grain samples.

Methods: a) Development has continued on the Electronic Grain Probe Insect Counter (EGPIC - U.S. Patent No.5,646,404 issued 7/97) that electronically counts the number of insects that fall through it as a means of monitoring infestations in stored-products. As a result of previous system malfunction problems encountered after phosphine fumigations during an earlier field test, the use of hermetically sealed electrical connectors and the encasement of all exposed conductors in silicon sealant was introduced. Another problem encountered in earlier field tests was extraneous insect counts due to electrical transients induced by mechanical jarring of the probe signal cables, so alternative cables were studied. A loss in counting accuracy after a change in sensor head material from PVC plastic pipe to black Delrin (DuPont, Wilmington, DE) because of its superior machinability led to a study of the detrimental effect of internal infrared-light reflections (diffusing the beam) in the sensor head.

Results: a) Laboratory phosphine tests performed on components in collaboration with Dr. James Leesch, ARS, Fresno, CA indicated no corrosion problems and an EGPIC System field test performed in collaboration with Dr. Thomas Phillips at Oklahoma State Univ., Stillwater, OK showed that the system operated with no detrimental effects during and after a phosphine fumigation. This result demonstrates the use of EGPIC in observing the efficacy of a fumigation in real-time without having to enter the storage bin. A new cable was selected with separate signal ground and braided shield components which did not result extraneous insect counts even when hit with a hammer. The study of internal infrared-light reflections in the sensor head led to incorporating an abrasion of its inner surface that resulted in improved performance beyond that previously observed with any material.

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DETECTION AND POPULATION ESTIMATION OF STORED PRODUCT INSECTS: SMARTS, ICE & ALFID

D. Shuman, N.D. Epsky, and R.W. Mankin

Objectives: To develop and evaluate automated systems for monitoring infestations in stored-products and for quantifying hidden infestations in grain samples.

Methods: a) Development has continued on the Serial Multiplexing Addressable Register Transmission System (SMARTS - U.S. Patent allowed) that can efficiently transmit data from over a million monitoring sensors (e.g., EGPIC, acoustic, temperature, etc.) distributed throughout a storage facility back to a central computer. A SMARTS Universal Node (SUN) circuit was developed to be the basic building block of any size network implementation.

b) Development has continued on the Insect Counting Electrocutor (ICE) that can automatically monitor the population dynamics of stored-product moths by electronically counting the number insects that are attracted into its electrocuting grid.

c) Development has continued on a new design for an acoustic infestation monitoring system suitable for large-scale applications. The system should employ a large array of acoustical sensors on cables, each capable of listening continuously for insect sounds and transmitting this data over long distances back to a central computer.

d) Development has continued on the Acoustic Location Fingerprinting Insect Detector (ALFID) System for grain sample inspection. An extension on the CRADA with DRT, Inc has allowed research to continue on an ALFID System employing the ultra-sensitive fluidic acousto-sensors.

Results: a) The Universal Node (SUN) printed circuit board layout was completed and manufactured to fit into custom made hermetically sealed chassis. It provides for jumper selectable options of transmission speed, numbers of inputs per node, and functional location within a SMARTS implementation. A ten-node system was assembled for field testing and performance validation.. SMARTS software development has incorporated many user selectable options such as a polling address list, polling period, and error detection criteria.

b) A patent application on the Insect Counting Electrocutor and its use with SMARTS was prepared and submitted to the U. S. Patent and Trademark Office. A variety of commercial inductive sensors were tested for ability to separate insect counts from induced power noise and pheromones were tested for attracting moths into the high-voltage grid.

c) An acoustic infestation monitoring system interface circuit was designed to locally detect, count, and store the number of sounds received by each acoustic sensor, and to interface this stored number with SMARTS for transmission back to a central computer.

d) An ALFID grain container containing 16 acousto-fluidic sensors and custom-made acoustic horns was constructed by DRT, Inc. and delivered to ARS. The electronic amplifiers for the acousto-fluidic sensor outputs had an inappropriate range for the ALFID interface circuit, and were redesigned for better compatibility.

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CENTER PUBLICATIONS LIST - OCTOBER 1997-SEPTEMBER 1998

Reprints may be requested by number

M-3193 SIVINSKI, J. M., ALUJA, M., and LOPEZ, M. 1997 September. Spatial and Temporal Distributions of Parasitoids of Mexican Anastrepha Species (Diptera: Tephritidae) within the canopies of Fruit Trees. Ann. Entomol. Soc. Am. 90: 604-618.

M-3194 SUTTON, B. D. and CARLSON, D. A. 1997 September. Cuticular Hydrocarbon Vairation in the Tbanidae (Diptera): Tabanus nigrovittatus Complex of the North American Atlantic Coast. Ann. Entomol. Soc. Am. 90: 542-549.

M-3195 VALLES, S. M., KOEHLER, P. G., and BRENNER, R. J. 1997 October. Antagonism of Fipronil Toxicity by Piperonyl Butoxide and S,S,S-Tributyl Phosphorotrithioate in the German Cockroach (Dictyiptera: Blattellidae). J. Econ. Entomol. 90: 1254-1258.

M-3196 HANDLER, A. M. and GOMEZ, S. P. 1997. P-Element Excision in Drosophila is Stimulated by Gamma-Irradiation in Transient Embroyonic Assays. Genetical Research Camb. 70: 75-78.

M-3197 VALLES, S. M. 1997 October. A Vacuum-Assisted Apparatus for Collection of Live German Cockroaches (Dictyoptera: Blattellidae) from the Field. J. Entomol. Sci. 32: 416-420.

M-3198 MOUNT, G. A., HAILE, D. G., and DANIELS, E. 1997 November. Simulation of Management Strategies for the Blacklegged Tick (Acari: Ixodidae) and the Lyme Disease Spirochete, Borrelia burgdorferi. J. Med. Entomol. 90: 672-683.

M-3199 LANDOLT, P. J. 1997 November. Cabbage Looper (Lepidoptera: Noctuidae) Fecundity Maximized by a Combination of Access to Water and Food, and Remating. Ann. Entomol. Soc. Am. 90: 783-789 .

M-3200 SPRAGUE, V. and BECNEL, J. J. 1998. Note on the Name-Author-Date Combination for the Taxon MICROSPORIDIES Balbiani, 1882, when Ranked as a Phylum. J. Invert. Path. 71: 91-94.

M-3201 REINERT, J. F., KAISER, P. E., and SEAWRIGHT, J. A. 1997 December. Analysis of the Anopheles (Anopheles) quadrimaculatus Complex of Sibling Species (Diptera: Culicidae) Using Morphological, Cyotological, Molecular, Genetic, Biochemical and Ecological Techniques in an Integrated Approach. J. Mosq. Control. Assoc. 13: 1-102.

M-3201 CARLSON, D. A., REINERT, J. F., BERNIER, U. R., SUTTON, B. D., and SEAWRIGHT, J. A. 1997 December. Analysis of the Cuticular Hydrocarbons Among Species of the Anopheles quadrimaculatus Complex (Diptera: Culicidae). J. Mosq. Control Assoc. 13: 103-111.

M-3201 REINERT, J. F. 1997 December. Bibliography of Anopheles quadrimaculatus Say sensu lato (Diptera: Culicidae). J. Mosq. Control Assoc. 13: 112-161.

M-3203 WEAVER, D. K., ZETTLER, J. L., WELLS, C. D., BAKER, J. E., BERTSCH, W., and THRONE, J. E. 1997 December. Toxicity of Fractionated and Degraded Mexican Marigold Floral Extract to Adult Sitophilus zeamais (Coleoptera: Curculionidae). J. Econ. Entomol. 90: 1678-1683.

M-3204 HOY, J. B., PORTER, S. D., and KOEHLER, P. G. 1997. A Microcomputer-Based Activity Meter for Multiple Animals. J. Neuroscience Methods 72: 183-188.

M-3205 YU, S. J. and VALLES, S. M. 1997 December. Carboxylamidase Activity in the Fall Armyworm (Lepidoptera: Noctuidae) and Other Lepidoptera, Orthoptera and Dictyoptera. J. Econ. Entomol. 90: 1521-1527.

M-3206 PARE, P. W. and TUMLINSON III, J. H. 1998. Cotton Volatiles Synthesized and Released Distal to the Site of Insect Damage. Phytochemistry 47: 521-526.

M-3207 HEATH, R. R., EPSKY, N. D., DUEBEN, B. D., RIZZO, J., and JERONIMO, F. 1997 December. Adding Methyl-Substitutes Ammonia Derivatives to a Food-Based Synthetic Attractant on Capture of Mediterranean and Mexican Fruit Flies (Diptera: Tephritidae). J. Econ. Entomol. 90: 1584-1589.

M-3208 TEAL, P. E. A. and NACHMAN, R. J. 1997. Prolonged Pheromonotropic Activity of Pseudopeptide Mimics of Insect Pyrokinin Neuropeptides After Topical Application or Injection into a Moth. Regulatory Peptides 72: 161-167.

M-3209 MITCHELL, E. R., TINGLE, F. C., NAVASERO-WARD, R. C., and KEHAT, M. 1997 December. Diamondback Moth (Lepidoptera: Plutellidae): Parasitism by Cotesia plutellae (Hymenoptera: Braconidae) in Cabbage. Fla. Entomol. 80: 477-489.

M-3210 RÖSE, U. S. R., LEWIS, W. J., and TUMLINSON III, J. H. 1998. Specificity of Systemically Released Cotton Volatiles as Attractants for Specialist and Generalist Parasitic Wasps. J. Chem. Ecol. 24: 303-319.

M-3211 BRENNER, R. J., MILNE, D. E., KINSCHERF, K. M., and CONNERS, T. F. 1998 February. Measuring Spatial Displacement of Blattella germanica (Blattaria: Blattellidae) Populations Pressured by Repellent-Treated Harborages. Environ. Entomol. 27: 10-21.

M-3212 VANDER MEER, R. K.; BANKS, W. A., and LOFGREN, C. S. 1998 February. Repellents for Ants (Patent). U.S. Patent, #5,721,274.

M-3213 WILLIAMS, D. F.; KNUE, G. J., and BECNEL, J. J. 1998. Discovery of Thelohania solenopsae from the Red Imported Fire Ant, Solenopsis invicta, in the United States. J. Invertebrate Pathology 71: 175-176.

M-3214 SIVINSKI, J. M., VULINEC, K., MENEZES, E. A., and ALUJA, M. 1998. The Bionomics of Coptera Haywardi (Ogloblin) (Hymenoptera: Diapriidae) and Other Pupal Parasitoids of Tephritid Fruit Flies (Diptera). Biological Control 11: 193-202.

M-3215 BARNARD, D. R., POSEY, K. H., SMITH, D., and SCHRECK, C. E. 1998. Mosquito Density, Biting Rate and Cage Size Effects on Repellent Tests. Medical and Veterinary Entomology 12: 39-45.

M-3216 CARPENTER, J. E. and GREANY, P. D. 1998. Comparative Development and Performance of Artificially Reared versus Host-Reared Diapetimorpha introita (Cresson) (Hymenoptera: Ichneumonidae) Wasps. Biological Control 11: 203-208.

M-3217 SIVINSKI, J. M., LLOYD, J. E., BESHERS, S. N., DAVIS, JR. L. R., SIVINSKI, R. G., WING, S. R., SULLIVAN, R. T., CUSHING, P. E., and PETERSSON, E. 1998. A Natural History of Pleotomodes Needhami Green (Coleoptera: Lampyridae): A Firefly Symbiont of Ants. Coleopterists Bulletin 52: 23-30.

M-3218 TEAL, P. E. A. 1998. Stimulation of Sex Pheromone Production in Corn Earworm Moths by Injection of Extracts of Heads of Males of the Caribbean Fruit Fly. Florida Entomol. 81: 98-104.

M-3219 BERNIER, U. R., CARLSON, D. A., and GEDEN, C. J. 1998. Gas Chromatography/Mass Spectrometry Analysis of the Cuticular Hydrocarbons from Parasitic Waps of the Genus muscidifurax. J. Amer. Soc. Mass Spectrometry 9: 320-332.

M-3220 PATZ, J. A., MARTENS, W. J. M., FOCKS, D. A., and JETTEN, T. H. 1998. Dengue Fever Epidemic Potential as Projected by General Circulation Models of Global Climate Change. Environ. Health Prospectives 106: 147-152.

M-3221 SIVINSKI, J. M., ALUJA, M., HOLLER, T. C., and EITAM, A. 1998. Phenological Comparison of Two Braconid Parasitoids of the Caribbean Fruit Fly (Diptera: Tephritidae). Environ. Entomol. 27: 360-365.

M-3222 COCKBURN, A. F. 1998. Insect Germplasm and Genome Databases. Amer. Entomol. 44: 16-19.

M-3223 BECNEL, J. J. and ANDREADIS, T. G. 1998. Ambloyospora salinaria n. sp. (Microsporidia: Amblyosporidae), Parasite of Culex salinarius (Diptera: Culicidae): Its Life Cycle Stages in an Intermediate Host. J. Invert. Pathol. 71: 258-262.

M-3224 SUITER, D: R., PATTERSON, R. S., and KOEHLER, P. G. 1998. Seasonal Incidence and Biological Control Potential of Aprostocetus hagenowii (Hymenoptera: Eulophidae) in Treehole Microhabitats. Environ. Entomol. 27: 434-442.

M-3225 ARBOGAST, R. T., WEAVER, D. K., KENDRA, P. E., and BRENNER, R. J. 1998. Implications of Spatial Distribution of Insect Populations in Storage ecosystems. Environ. Entomol. 202-216.

M-3226 LANDOLT, P. J., JEANNE, R. L., and REED, H. C. 1998. Chemical Communication in Social Wasps (Book Chapter). In R.K. Vander Meer, M. Breed, M. Winston, and K.E. Epselie [Eds.], Pheromone Communication in Social Insects. Westview Press, Boulder, CO; 216-235.

M-3227 BARNARD, D. R. 1998. Mediation of Deet Repellency in Mosquitoes (Diptera: Culicidae) by Species, Age, and Parity. J. Med. Entomol. 35: 340-343.

M-3228 DYBY, S. D. 1998. Method for Visualizing the Tracheal System of Newly Hatched Insects. Ann. Entomol. Soc. Am. 91: 350-352.

M-3229 UNDEEN, A. H. 1998. Microsporidia (Protozoa): A Handbook of Biology and Research Techniques. Weed Management Systems Handbook.

M-3230 POSEY, K. H., BARNARD, D. R., and SCHRECK, C. E. 1998. A Triple Cage Olfactometer for Evaluating Mosquito (Diptera: Culicidae) Attraction Responses. J. Med. Entomol. 35: 330-334.

M-3231 MAYER, M. S. and MITCHELL, E. R. 1998. Rapid Measure of Sex Pheromone Emission from Plastic Rope Dispensers: Example of Utility in Sexual Communication Disruption of the Diamondback Moth, Plutella xylostella. Phytoparasitica 26: 117-125.

M-3232 OBERLANDER, H., SILHACEK, D. L., and LEACH, C. E. 1998. Interactions of Ecdysteroid and Juvenoid Agonists in Plodia interpunctella (Hubner). Archives of Insect Biochem. & Phsiol. 38: 91-99.

M-3233 BARNARD, D. R., HARMS, R. H., and SLOAN, D. R. 1998. Biodegradation of Poultry Manure by House Fly (Diptera: Muscidae). Environ. Entomol. 27: 600-605.

M-3234 FARKAS, R., HOGSETTE, JR. J. A., and BORZSONYI, L. 1998. Development of Hydrotaea aenescens and Musca domestica (Diptera: Muscidae) in Poultry and Pig Manures of Different Moisture Content. Environ. Entomol. 27: 695-699.

M-3235 FRYAUFF, D. J., SHOUKRY, M. A., WASSEF, H. Y., GRAY, G., and SCHRECK, C. E. 1998. Contact Toxicity of Permethrin-Impregnated Fabric to Hyalomma anatolicum excavatum (Acari: Ixodidae): Effects of Laundering and Exposure and Recovery Times. J. Med. Entomol. 35: 335-339.

M-3236 OBERLANDER, H. and SILHACEK, D. L. 1998. New Perspectives on the Mode of Action of Benzoylphenyl Urea Insecticides. Applied Agriculture: Insecticides with Novel Modes of Action [Eds. I. Ishaaya and D. Degheele); 92-105.

M-3237 SHIRK, P. D., BROZA, R., HEMPHILL, M., and PERERA, O. P. 1998. α-Crystallin Protein Cognates in Eggs of the Moth, Plodia interpunctella, possible chaperones for the follicular epithelium yolk protein. Insect Biochem. & Molecular Biol. 28: 151-161.

M-3238 JENSEN, T., COCKBURN, A. F., and BARNARD, D. R. 1997. Tale of Two Species: The Interdependence of Molecular Genetic and Biological Studies for Characterizing Members of the Anopheles quadrimaculatus and Anopheles crucians Species Complexes (Abstract). 1997 Proc. Mosq. and Vector Control Assoc. of CA; 65: 33-34.

M-3239 HANDLER, A. M., MCCOMBS, S. D., FRASER, M. J., and SAUL, S. H. 1998. The Lepidopteran Transposon Vector, Piggybac, Mediates Germ-line Transformation in the Mediterranean Fruit Fly. Proc. Natl Acad. of Sci. 95: 7520-7525.

M-3240 ROMERO, M. I. and BRENNER, R. J. 1998. Instrumentation and Measurement of Airflow and Temperature in Attics Fitted with Ridge and Soffit Vents. Ashrae Transactions 1998, V. 104, Pt. 2. (p. 1-10); American Society of Heating, Refrigerating and Air-Conditioning Engineers, Inc. (ASHRAE).

M-3241 GEDEN, C. J., BERNIER, U. R., CARLSON, D. A., and SUTTON, B. D. 1998. Identification of Muscidifurax spp., Parasitoids of Muscoid Flies, by Composition Patterns of Cuticular Hydrocarbons. Biological Control 12: 200-207.

M-3242 WARTHEN, J. D., MITCHELL, E. R., and HARTE, E. M. 1998. Laboratory Release Rate Studies of Shin-Etsu, Freshly Manufactured Versus Cold-Stored, Fresh Pheromone Ropes. J. Environ. Sci. Health A33: 701-714.

M-3243 VALLES, S. M. 1998. Stage-Dependent Bendiocarb Tolerance in the German Cockroach (Dictyoptera: Blattellidae). J. Entomol. Sci. 33: 313-315.

M-3244 VANDER MEER, R. K. 1997. The Chemical Ecology of Fire Ants: Gladular, Chemical, and Behavioral Parsimony (Abstract). C.N.R.S. - Conference Jacques Monod; Chemical Communication in Vertebrates and Invertebrates: Evolution, Function and Perception of Communication Substances and Evoked Responses; E. D. Morgan and G. Sicard [eds.]: 119-120.

M-3245 GUPTA, P. and FERKOVICH, S. M. 1998. Interaction of caly fluid and venom from Microplitis croceipes (Braconidae) on developmental disruption of the natural host, Heliocoverpa zea, and two atypical hosts, Galleria mellonella and Spodoptera exigua. J. Insect Physiology 44: 713-719.

M-3246 BRENNER, R. J., FOCKS, D. A., ARBOGAST, R. T., WEAVER, D. K., and SHUMAN, D. 1998. Practical Use of Spatial Analysis in Precision Targeting for Integrated Pest Management. Amer. Entomol.: 79-101.

M-3247 BOROVSKY, D. and CARLSON, D. A. 1998. Materials and Method for Control of Pests. U.S. Patent 5,792,750.

M-3248 CRESPO, D. C., LECUONA, R. E., and HOGSETTE, JR. J. A. 1998. Biological Control: An Important Component in Integrated Management of Musca domestica (Diptera: Muscidae) in Caged-Layer Poultry Houses in Buenos Aires, Argentina. Biological Control 13: 16-24.

M-3249 BERNIER, U. R., CARLSON, D. A., and GEDEN, C. J. 1998. Gas Chromatographic/Mass Spectrometric Analysis of the Cuticular Hydrocarbons from Parasitic Wasps of the Genus Spalangia (Abstract). Pittcon '98: A Kaleidoscope of Chemistry; New Orleans, LA; March 15, 1998; #633.

M-3250 BOOTH, M. M., YOST, R. A., BERNIER, U. R., KLINE, D. L., and BARNARD, D. R. 1998. GC/MS Analysis of Human Skin Emanations (Abstract). Pittcon '98: A Kaleidoscope of Chemistry; New Orleans, LA; March 1-5, 1998; #582.

M-3251 BURKETT, D. A., BUTLER, J. F., and KLINE, D. L. 1998. Field Evaluation of Colored Light-Emitting Diodes as Attractants for Woodland Mosquitoes and Other Diptera in North Central Florida. J. Mosq. Control. Assoc. 14: 186-195.

M-3252 HEATH, R. R. and DUEBEN, B. D. 1998. Analytical and Prepartive Gas Chromatography (Book Chapter). Methods in Chemical Ecology: Chemical Methods; J.G. Millar and K.F. Haynes [eds.]; Kluwer Academic Publishers; 1998: 1: 85-126.

M-3253 HEATH, R. R. and EPSKY, N. D. 1998. Trapping System for Mediterranean Fruit Flies. U. S. Patent, 5,766,617.

M-3254 CARLSON, D. A., OFFOR, I. I., MESSOUSSI, S. E., MATSUYAMA, K., MORI, K., and JALLON, J. M. 1998. Sex Pheromone of Glossina tachinoides: Isolation, Identification, and Synthesis. J. Chem. Ecol. 24: 1563-1575.

M-3255 DONG, K., VALLES, S. M., SCHARF, M. E., ZEICHNER, B., and BENNETT, G. W. 1998. The Knockdown Resistane (KDR) Mutation in Pyrethroid-Resistant German Cockroaches. Pesticide Biochemistry and Physiology 60: 195-204.

M-3256 DE MORAES, C. M., LEWIS, W. J., PARE, P. W., ALBORN, H. T., and TUMLINSON III, J. H. 1998. Herbivore-infested Plants Selectively Attract Parasitoids. Nature 393: 570-573.

M-3257 BAAKRI, A., HADIS, H., EPSKY, N. D., HEATH, R. R., and HENDRICKS, J. 1998. Female Ceratitis capitata (Diptera: Tephritidae) Capture in a Dry Trap Baited with a Food-Based Synthetic Attractant in an Argan Forest in Moroco. Part I. Low Population Field Test. Teh Canadian Entomologist 130:349-356.

M-3258 NACHMAN, R. J., TEAL, P. E. A., and HOLMAN, G. M. 1998. Mimetic Insect Pyrokinin Analogs For Insect Control. U.S. Patent 5,795,857.

M-3259 CONN, J. E., MITCHELL, S. E., and COCKBURN, A. F. 1998. Mitochondrial DNA analysis of the neotropical malaria vector Anopheles nuneztovari. Genome 41: 313-327.

M-3260 MOSER, B. A., BECNEL, J. J., MARUNIAK, J., and PATTERSON, R. S. 1998. Analysis of the Ribosomal DNA Sequences of the Microsporidia Thelohania and Vairimorpha of Fire Ants. J. Invert. Pathol. 72: 154-159.

M-3261 VALLES, S. M., SANCHEZ-ARROYO, H., BRENNER, R. J., and KOEHLER, P. G. 1998. Temperature Effects on Lambda-Cyhalothrin Toxicity in Insecticide-Susceptible and Resistant German Cockroaches (Dictyoptera: Blattellidae). Fla. Entomol. 81: 193-201.

M-3262 BRENNER, R. J., MILNE, D. E., and HEDGES, S. A. 1998. Biological Vacuum Device to Enhance Environmental Quality. U. S. Patent, 5,806,238.

M-3263 FONAGY, A., TEAL, P. E. A., MEREDITH, J. A., KORMENDY, C., and TUMLINSON III, J. H. 1998. Partial Identification of a New Pheromonotropic Peptide from the Mamestra brassicae. Annals of the New York Academy of Sciences 839: 488-490.

M-3264 GEDEN, C. J., ARRENDS, J. J., RUTZ, D. A., and STEINKRAUS, D. C. 1998. Laboratory Evaluation of Beauveria bassiana (Moniliales: Moniliaceae) Against the Lesser Mealworm, Alphitobius diaperinus (Coleoptera: Tenebrionidae) in Poultry Litter, Soil and a Pupal Trap. Biological Control 13: 71-77.

M-3265 VANDER MEER, R. K. and SHATTERS, R. G. 1998. The Effect of Fire Ants on Soybean Germination and Growth. Tenth International Symposium on Insect-Plant Relationships; July 4-11, 1998; Oxford; p. 62.

M-3266 CARLSON, D. A. 1998. What Mosquitoes Smell These Days. BioScience 48: 961-963.

M-3267 LAHAV, S., SOROKER, V., VANDER MEER, R. K., and HEFETZ, A. 1998. Nestmate Recognition in the Ant Cataglyphis niger: Do Queens Matter? Behav. Ecol. Sociobiol. 43: 203-212.

M-3268 MICIELI, M. V., GARCIA, J. J., and BECNEL, J. J. 1998. Horizontal Transmission of Amblyospora dolosi (Microspora: Amblyosporidae) to the Copepod Metacyclops mendocinus (Wierzejski, 1892). J. Invert. Pathol. 72: 330-335.

M-3269 BARNARD, D. R. 1998. Arthropod-Related Occupational Health Problems. Encyclopaedia of Occupation Health and Safety; 70.9-70.10.

M-3270 MYERS, M. L. and BARNARD, D. R. 1998. Beekeeping, Insect Raising and Silk Production. Encyclopaedia of Occupational Health and Safety; 70.34-70.38.

M-3271 VALLES, S. M., OSBRINK, W. L., OI, F. M., and BRENNER, R. J. 1998. Cytochrome P450 Monooxygenase Activity in the Dark Southern Subterranean Termite (Isoptera: Rhinotermitidae). J. Econ. Entomol. 91: 1131-1135.

M-3272 KLINE, D. L. and MANN, M. O. 1998. Evaluation of Butanone, Carbon Dioxide and 1-octen-3-ol as Attractants for Mosquitoes Associated with North Central Florida Bay and Cypress Swamps. J. Mosq. Control Assoc. 14: 289-297.

M-3273 WILLIAMS, D. F. and OI, D. H. 1998. Impact of a Microsporidian Pathogen, Thelohania solenopsae, on Red Imported Fire Ants. Proc. of the Imported Fire Ant Research Conference, April 1998: 79-86.

M-3274 VANDER MEER, R. K. and ALONSO, L. E. 1998. Newly Mated Queen Adoption and Polygyny. Proc. of the Imported Fire Ant Research Conference, April 1998: 118.

M-3275 VANDER MEER, R. K. and ALONSO, L. E. 1998. Adoption of Newly Mated Queens: What are the Consequences for Control. Proc. of the Imported Fire Ant Research Conference, April 1998: 120.

M-3276 MOUNT, G. A. 1998. A Critical Review of Ultralow-Volume Aerosols of Insecticide Applied with Vehicle-Mounted Generators for Adult Mosquito Control. J. Mosq. Control Assoc. 14: 305-334.

M-3277 OI, D. H., WILLIAMS, D. F., BRIANO, J. A., and CALCATERRA, L. A. 1998. Research Update On Solenopsis (Labauchena) daguerrei, a Parasitic Ant of Imported Fire Ants. Proc. of the Imported Fire Ant Research Conference, April 1998: 129-130.

M-3278 EPSKY, N. D. and HEATH, R. R. 1998. Exploiting the Interactions of Chemical and Visual Cues in Behavioral Control Measures for Pest Tephritid Fruit Flies. Fla. Entomol. 81: 273-282.

M-3279 WOJCIK, D. P. and BRENNER, R. J. 1998. Bait Studies on Fire Ants & Fire Ant Toxicants on Texas Cave Crickets & Fish-Bait Crickets. Proc. of the Imported Fire Ant Research Conference, April 1998: 142.

M-3280 WOJCIK, D. P., BRENNER, R. J., FOCKS, D. A., WILLIAMS, D. F., and ALLEN, C. R. 1998. Impact of the Imported Fire Ant on Biodiversity: Standardized Spaital Monitoring of Foraging Interactions. Proc. of the Imported Fire Ant Research Conference, April 1998: 169-174.

M-3281 PORTER, S. D. 1998. Biology and Behavior of Pseudacteon decapitating Flies (Diptera: Phoridae) that Parsitize Solenopsis Fire Ants (Hymenoptera: Formicidae). Fl. Entomol. 81:292-309.

M-3282 PORTER, S. D. 1998. Host-Specific Attraction of Pseduacteon Flies (Diptera: Phoridae) to Fire Ant Colonies in Brazil. Fla. Entomol. 81: 423-429.

M-3283 SIVINSKI, J. M. 1998. Phototropism, Bioluminescence and the Diptera. Fla. Entomol. 81: 282-292.